AMENDMENTS TO THE CLAIMS:

09/591,789

This listing of the claims will replace all prior versions, and listings, of the claims in the present application.

5911990 6/15/99 (02Ce) RFD: 8/13/96

Listing of Claims:

MOUNTED 5/4/06

Claims 1-6 (canceled).

7 (currently amended). A method to increase production of at least one Th1 cytokine or to decrease production of at least one Th2 cytokine, comprising ~~in an individual free of infection with an immunodeficiency-type retrovirus comprising~~ administering to a human an effective amount of a peptide to increase production of at least one Th1 cytokine or to decrease production of at least one Th2 cytokine, said peptide consisting of the amino acid sequence Cys Lys Pro Ile Ser Gly His Asn Ser Leu Phe Trp Tyr Arg Gln Thr (SEQ ID NO:1), C K P I S G H N S L F W Y R Q T and which human ~~to an human individual~~ is free of infection with an immunodeficiency-type retrovirus ~~in an amount sufficient to increase production of at least one Th1 cytokine or decrease production of at least one Th2 cytokine~~.

IMMUNOL 1996 87(2):198
J.I. 1995 155(4):2282
IMMUNOL 1998 99(4):462

TCR CDR1 VB 8.1

Claims 8-23 (canceled).

INDUCES Th1 BY ↑ IL-2

24 (previously presented). The method according to claim 7 in which the Th1 cytokine is selected from the group consisting of interleukin 2 and interferon-γ.

25 (currently amended). The method according to claim 7 in which the Th2 cytokine is selected from the group consisting of interleukin-4, interleukin 5, interleukin 6, and interleukin 10 ~~and immunoglobulin G~~.

26 (new). A method to increase production of at least one Th1 cytokine or to decrease production of immunoglobulin G, comprising administering to a human an effective amount of a peptide to increase production of at least one Th1 cytokine or to decrease

3

TCRs / T-CELL RECEPTOR 2
AUTOANTIBODIES
CDR

3.

production of immunoglobulin G, said peptide consisting of the amino acid sequence Cys Lys Pro Ile Ser Gly His Asn Ser Leu Phe Trp Tyr Arg Gln Thr (SEQ ID NO:1), and which human is free of infection with an immunodeficiency-type retrovirus.

Id. at 1441 (emphasis in the original). Further, the *Brana* Court made clear that the Patent and Trademark Office has the initial burden of challenging a presumptively correct assertion of utility; evidence must be presented that those of skill in the art would doubt the disclosure. Only then must the applicant provide rebuttal evidence.

Further, the Federal Circuit in *Brana* explained that even if one of skill in the art would have questioned the asserted utility, all applicants need do to overcome the rejection is to proffer sufficient evidence to convince one skilled in the art of the asserted utility. *Id.* at 1441.

In the present invention, Applicants have provided such evidence. The Examiner's attention is again directed to U.S. Patent No. 5,911,990 to Marchalonis *et al.*, (Reference AA, of record), which clearly teaches that administration of the peptide of SEQ ID NO:1 to a mouse suffering from murine AIDS is able to restore normal levels of Th1 and Th2 cytokines (increase production of a Th1 cytokine and decrease levels of a Th2 cytokine) (see Abstract). Applicants note that the mouse model is an art accepted model for testing compounds for their effect in humans. The Examiner has provided no justification for dismissing the results of this study.

The Examiner's attention is also invited to Sepulveda *et al.*, 2003, J. Cardiovasc. Pharmacol. 41:489-497, ("Sepulveda I", Reference AF, of record). Sepulveda I also teaches that administration of the peptide of SEQ ID NO:1 to a mouse infected with LP-BM5 results in a longer progression time to mADIS by increasing production of at least one Th1 cytokine is increased or decreasing production of at least one Th2 cytokine. Further, in mice infected with both LP-BM5 and with coxsackievirus CVB3, which leads to myocarditis, Sepulveda I discloses that administration of the peptide of SEQ ID NO:1 provides a protective effect against the development of said myocarditis. See Sepulveda I, page 7, left column. Thus, a nexus between increasing production of at least one Th1 cytokine is increased or decreasing production of at least one Th2 cytokine and AIDS and/or

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Synthetic Autoantigens of Immunoglobulins and T-Cell Receptors: Their Recognition in Aging, Infection, and Autoimmunity (43801)

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VB8.1 TCR CDR PEPTIDES; NO DEMONSTRATION THAT IT CAN MODULATE TH1/TH2 PROFILES.

Abstract. Immunoglobulins and their close relatives, the antigen-specific T-cell receptors, are recognition proteins that express structures which readily serve as self-immunogens. Healthy humans can produce antibodies against variable region-defined recognition structures termed idiotypes, as well as against constant region structures, and the levels of these can increase markedly in autoimmune disease; e.g., rheumatoid factors are autoantibodies directed against a conformational determinant of the γ heavy chain. More recent analyses employing synthetic peptide technologies and construction of recombinant T-cell receptors document that autoantibodies directed against both variable and constant region markers of the α/β T-cell receptor occur in healthy individuals. Alterations in levels of antibody, usage of IgM or IgG isotypes, and specificity for particular peptide-defined regions vary with natural physiological processes (aging, pregnancy), with artificial allografting, with retroviral infection, and with the inception and progression of autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus). Two of the major autoimmunogenic regions of the Tcr α/β are "constitutive" markers inasmuch as all individuals tested produce antibodies against these regions. The most frequently observed autoantibodies are against Tcr Vβ CDR1 and Fr3 markers. It is hypothesized that these are normally involved in immunoregulation. Autoantibodies usually are not detected against CDR2 region determinants, or the "private idiotypes" defined by the CDR3 region, or the highly conserved FR4 segment specified by the joining gene segment. However, autoantibodies against the CDR2 of the Tcrα chain occur in some SLE patients, and healthy pregnant women produce antibodies against the common peptide determinant expressed by the joining gene and the beginning of the Cα or Cβ domain. Although the precise role of the naturally occurring autoantibodies in immunoregulation remains to be determined, modification of the course of autoimmune diseases in experimental rodent models (experimental allergic encephalomyelitis) has

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[2] Kaymaz H, Wang AC, Marchalonis JJ, unpublished.

0037-9727/94/2072-0129$10.50/0

been successfully carried out by immunization with synthetic peptides corresponding to the CDR2 and Fr3/CDR3 segments, and immunization of humans with synthetic Vβ CDR2 segments may prove helpful in multiple sclerosis. Moreover, infusion of intravenous immunoglobulins has been successful in the treatment of many autoimmune diseases, including examples where levels of T cells bearing particular Vβ gene subsets were elevated. The recent knowledge gained from T-cell receptor structural analysis and antigenic modeling holds promise for determining the roles of particular variable domain structures in antigen recognition MHC-restriction and immunoregulation, and in the development of synthetic and recombinant reagents for modulation of autoimmune and infectious diseases. [P.S.E.B.M. 1994, Vol 207]

Immunoglobulins and their close relatives the α/β T-cell receptors are examples of syngeneic proteins that express structures which readily serve as self-immunogens. These are the combining site-defined markers or idiotypes that are recognized by T cells (1–3) and induce the formation of anti-idiotypic antibodies (4–6) that serve as part of a regulatory network (7, 8). Studies with antibodies have been carried out with a great degree of precision because of the availability of large quantities of monoclonal protein and the application of hybridoma technology coupled with accurate 3-dimensional characterization by x-ray crystallography (9, 10). The situation with respect to T-cell receptors is less clear because they have not yet been produced in sufficient quantity even though gene sequence is available (11, 12) to allow antigenic or 3-dimensional characterization of the intact proteins. In our initial studies, we approached the problem of antigenic and structural characterization of T-cell receptors by focusing upon the sequence of the Tcr β chain which is homologous to Ig λ light chain (11–13). Our objectives were (i) to use computer analyses of sequences and predicted structures to identify a "universal" peptide antigen shared between the variable domains of Tcrs and light chains (14–17); and (ii) to use comprehensive peptide synthesis (18) to duplicate the complete covalent structure of a human Tcr β chain (19, 20). These peptides served as the basis for mapping epitopes shared between Tcrs and Ig-L chains (20, 21). In order to calibrate the procedures, we began by duplicating the sequence of the human monoclonal λ light chain Mcg by a nested set of overlapping synthetic 16-mer peptides and mapping epitopes recognized by xenoantisera (21) and natural human autoantibodies (22). The availability of milligram quantities of intact λ chain of known structure allowed us to test for the binding of antipeptide antibodies to the native structure. We used competitive inhibitions to ascertain the degree to which conformations of peptides in solution mimicked the antigenic structures of the same sequences in the intact structure. Parallel approaches to investigate the reactivities of anti-Tcr peptides with intact molecules were carried out using Western blot analyses (23), immunocytofluorescence with monoclonal human T cells, and the construction of a recombinant single chain Vα/Vβ structure.

The tie-in of the synthetic antigen approaches to autoimmunity and immunoregulation arose in our hands from control experiments in which sera of unimmunized humans (19, 22), mice, and rabbits were tested for IgG binding to the overlapping sets of peptides duplicating Tcrβ (19) and Igλ chains (22). The normal IgG pools of these species bound to a limited number of peptides, and these corresponded to the same sequence regions in the β and λ chains. Natural autoantibodies occur in diverse vertebrate species including sharks (24), bony fishes (25), mice (26), and humans (27). These autoantibodies react with a large set of self-antigens such as DNA, thyroglobulin, cytoskeletal proteins, the senescent cell antigen (28), and the constant regions of IgG heavy chain (29). Thus, it was worthwhile to characterize the autoantibodies to Tcrs in healthy individuals and in those with autoimmune disease to determine the parameters of expression of these antibodies and to elucidate their possible functions in immunoregulation. The importance of such autoantibodies in clinical medicine is suggested by the successful use of intravenous immunoglobulins as therapy for Kawasaki's disease (30), multiple sclerosis (31), acute idiopathic thrombocytopenic purpura (32, 33), and experimental rodent autoimmune uveoretinitis (34). It can be hypothesized that the diseases are ameliorated by the action of autoantibodies specific for individual sets of Tcr domains that modify the expression or function of T cells bearing these particular Vβ regions (35).

We will use the data available on the structure of immunoglobulins and on the construction of synthetic and recombinant idiotypes (3, 4, 36–40) in conjunction with recent modeling of Tcrs (17, 20, 41–44) to provide a framework for interpreting the antigenic and functional structures of Ig/Tcr V and C domains. The levels and isotype expression (IgM or IgG) of autoantibodies to Tcr peptide-defined regions are dependent upon the following conditions: aging, autoimmune disease, allograft transplantation, pregnancy, and retroviral in-

Table I. Salient Autoantigenic Peptide Segments

	Synthetic sequence	Description
β3	C K P I S G H N S L F W Y R Q T	CDR1/Fr2; public Vβ "idiotope"; major autoantigenic site
β8	K I Q P S E P R D S A V Y F C A	Fr3; major autoantigenic site; marker for Vβ gene families
β17	Q P L K E Q P A L N D S R Y C L	Cβ large loop in man; N-glycosylation site; major autoantigen
β5	L L I Y F N N N V P I D D S G M	CDR2; low levels of autoantibodies in normals; rabbit antibodies bind strongly to T cells and SCVαVβ; public Vβ idiotope
α5	L L L K Y T S A A T L V K G I N	CDR2; Public Vα idiotope; major autoantigen in SLE; minimal autoantibody in normals
Jβ	A N Y G Y T F G S G T R L T V V	Cross-reactive set containing the Fr4 segment specified by the joining gene segment. Mimicked by the constant segment within the HIV-1 V3 loop
Jα	S A S K I I F G S G T R L S I R	
Mcg10	V F G T G T K V T V L G Q P K A	
α11	R L S I R P N I Q N P D P A V Y	"Switch peptide" continuous with Fr4
β11	T R L T V V E D L N K V F P P E	Usually not autoantigenic; autoantibodies found in pregnancy

sider this region to constitute a public idiotope restricted to particular Vβ gene subgroups. Only low levels of autoantibodies, if any, to the corresponding segment of the α chain (α5) occur in most human sera. However, sera from SLE patients from North Carolina have elevated levels of IgG antibodies to this peptide by comparison with normal healthy women who are pregnant (51).

The set of peptides (α10, 11; β10, 11; Mcg10) containing the Fr4 segment that is encoded by the joining region gene of Tcrs and light chains is of interest because the FGXGT+L motif is highly conserved in Tcrs and light chains of species widely spread in vertebrate evolution (13, 15, 17). Usually, there is little detectable natural reactivity directed against this region of the molecule. In fact, we have been unable to immunize rats and mice with this peptide (16). Rabbits respond quite well because they express light chains with a negatively charged glutamic acid (E) at Position 12, as opposed to positively charged arginine (R) or lysine (K) residues. Rabbit antibodies directed against the synthetic Jβ peptide cross react in ELISA with the Jα and Jλ peptides illustrated here.

We have recently found that IgG preparations from plasma of humans infected with HIV-1 contain antibodies directed against the Jβ segment (52). This is because there is a conserved stretch of the form GPG*RAF (Y or V) in the major neutralizing determinant V3 loop of the gp120 glycoprotein of HIV-1 (53). As illustrated in Figure 3, the conserved V3 peptide is sufficiently similar to the Tcr peptide to cause the generation of antibodies by antigenic mimicry (52). In addition to the J peptides, pregnant women have autoantibodies directed against the α11 and β11 peptides that overlap the Jβ and V3 segment but continue through the "switch region" into the constant domain.

Structural and Functional Interpretation of Autoantigenic Sites on Tcrs

Mice and other species contain natural antibodies directed against V_H peptides of the phosphorylcholine binding myeloma protein TEPC 15 (54). Antibodies to human Tcr occur in the natural alloimmunization process during pregnancy (55, 56) and in renal transplantation (57). The results of epitope mapping using comprehensive peptide synthesis facilitate the localization of these determinants. Antibodies against Tcr Vβ8.2 appear in B10.pL mice in the recovery phase of exper-

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Vβ8.1 TCR CDR1 PEPTIDE TAUGHT
HIV-1 PATIENTS MAKE AABs TO THIS PEPTIDE

Analysis of Autoantibodies to T-Cell Receptors among HIV-Infected Individuals: Epitope Analysis and Time Course

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* NO RECOGNITION THAT Th1/Th2 RESPONSES CAN BE MODIFIED

Individuals seropositive for human immunodeficiency virus type 1 (HIV) express elevated levels of autoantibodies (AAbs) directed against recombinant T-cell receptors (TCRs) and synthetic peptide epitopes duplicating β chain markers. We performed longitudinal studies of anti-TCR AAbs in HIV-1-infected individuals, making comparisons with uninfected sera and sera from other individuals infected with a nonviral agent. We determined levels of autoantibodies by titration using enzyme-linked immunosorbent assay (ELISA) and developed a means for characterizing "autoantibody CDR recognition spectrotypes" for individual sera. Antibody levels against certain defined synthetic epitopes were substantially elevated in HIV-infected subjects relative to reactivities by control groups. Individual sera showed relatively high AAb levels to a subset of CDR1 peptide epitopes. Two patients who subsequently developed AIDS showed particular reactivity to Vβ2.1, 8.1, 10.1, and 22.1 epitopes. Our results show that production AAbs to TCR Vβ epitopes is a general consequence of HIV infection. The response is individual but shows some restriction and shifts in AAb subpopulations often occur with time. © 1997 Academic Press

INTRODUCTION

One of the major consequences of infection with human immunodeficiency virus type 1 (HIV) is the elaboration of autoimmune reactivity similar to that found in graft versus host disease (1) or systemic lupus erythematosus (2). In particular, autoimmune reactivity has been found against MHC antigens (3, 4), T-cell receptors (5, 6), and the Fab fragments of immunoglobulin (7–9) as well to a number of other autoantigens (2, 10–12). We recently reported that preparations of pooled IgG immunoglobulin isolated from the plasmas of HIV-infected individuals contain autoantibodies directed against recombinant T-cell receptor Vα/Vβ constructs and defined peptide epitopes corresponding to the CDR1 and FR3 segments of the T-cell receptor (TCR) Vβ chain (5). It was suggested that these autoantibodies arise from a deregulation of the immune system involving the production of autoantibodies against "public idiotopes" of T-cell receptor variable regions. In addition, antibodies reactive with a peptide epitope from the V3 loop of gp120 of HIV were present, and these cross-reacted with T-cell receptors because of shared sequence and antigenic homology (antigenic mimicry) to a sequence defined by the joining segment of the T-cell receptor β chain (5).

Here, we analyze the autoantibody reactivities to the recombinant TCR Vα/Vβ construct and peptide epitopes of the Vβ domain of ten HIV-seropositive individuals in a longitudinal study over a period of 2 years. The goals of the study were to characterize reactivities of individual patients and determine the early time course of anti-TCR autoantibody production in HIV infection. Since such autoantibodies are polyclonal (13), we devised a procedure to estimate the relative contribution in each individual of autoantibodies against products of distinct TCR Vβ gene products. To accomplish this, we derived an "autoantibody CDR1 (or FR3) recognition spectrotype" for each serum by quantifying the reactivity against a set of synthetic 16-mer peptides, each of which corresponds to the product of a separate Vβ gene. We provide evidence that the levels of IgG autoantibodies to the intact recombinant TCR and the characteristic peptide epitopes are elevated at first analysis of the seropositive individuals, and that high autoantibody levels are maintained throughout the 2-year period. Autoantibody recognition spectrotype analysis disclosed that HIV-infected individuals have individual profiles but that these have common characteristics distinguishing them from either uninfected, asymptomatic individuals or patients infected with a separate agent, the fungus *Coccidioides immitis*.

MATERIALS AND METHODS

Human sera and IgG. Sera of clinically healthy individuals of both sexes in the age range of 20–60 years

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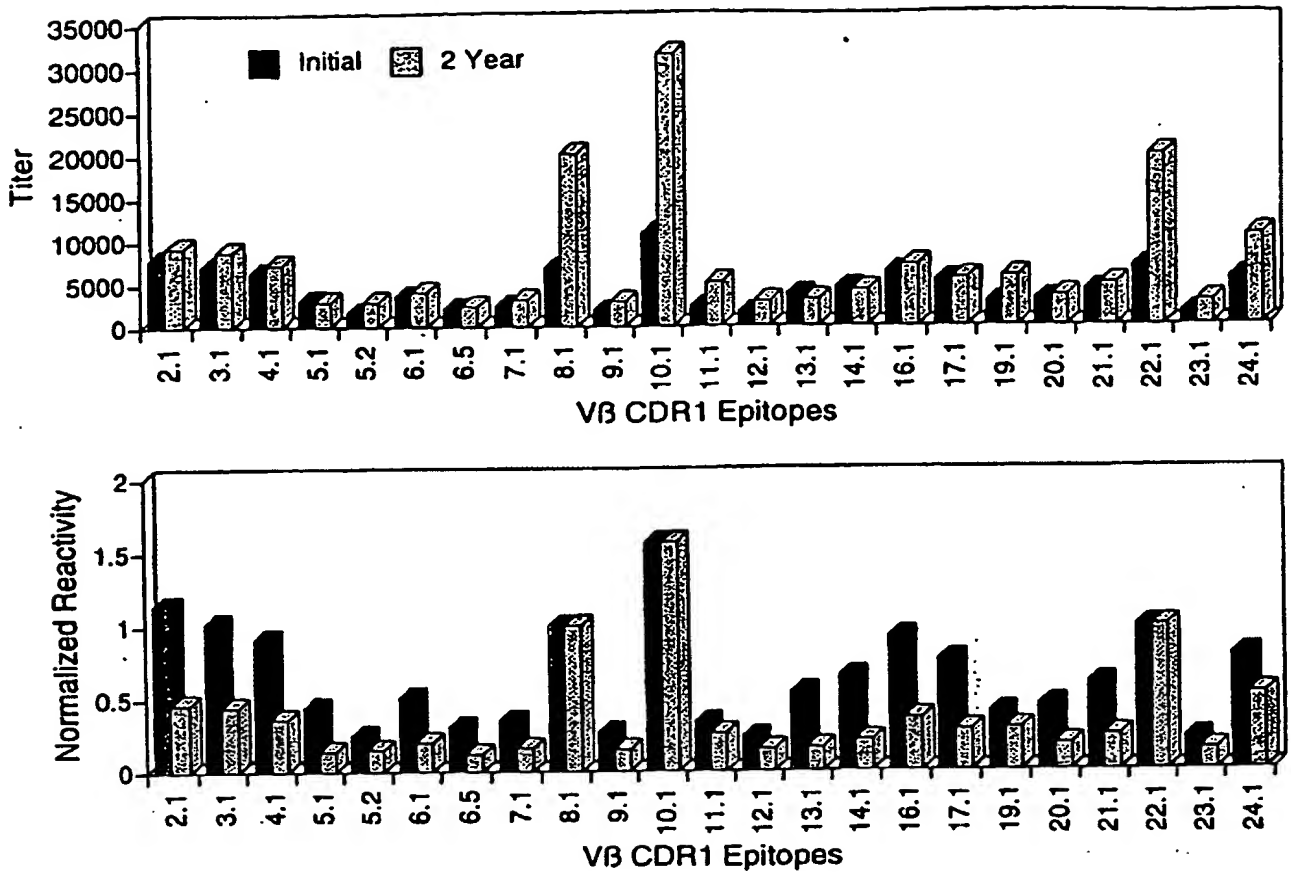

FIG. 9. Autoantibody CDR1 recognition spectratype of patient 1018 comparing values at initial presentation and at 2 years. (Top) Data as binding titer. (Bottom) Data normalized with the reactivity with Vβ8.1 CDR1 defined as 1.0.

this specificity do not occur in nonretrovirally infected humans (14). Rabbits by contrast, produce high titer antibodies to conjugates of the Jβ peptide (31) which bind to human T-cells (32) and can block *in vitro* infection of T-cells by live HIV (J. J. Marchalonis, S. F. Schluter, and R. T. Kennedy, unpublished observations).

The Vβ-CDR1 epitopes are defined completely by the individual Vβ gene and can, thus, be considered "public idiotopes" (14, 33). We have used this property to define autoantibody CDR1 recognition spectrotypes by constructing a set of homologous peptides, each of which corresponds to the product of an individual Vβ gene, and determining the autoantibody profiles of individual and sets of patients. The assays were performed by ELISA and incorporated both quantitative levels based upon titers or modified sum absorbancy and normalized comparisons with the reactivity to the Vβ8.1 peptide to illustrate the relative contributions of subsets of autoantibodies of distinct Vβ-CDR1 peptide results from an unusual process of immunoregulation because all mammals tested including humans (14), mice (34), rabbits, and lower primates (J. J. Marchalonis and A. Garza, unpublished observations) have naturally occurring autoantibodies. These peptides lack ostensive homology to viral peptides. Although rabbits are excellent antibody producers to most peptides in our comprehensive peptide epitope mapping set (14, 20), immunization with the Vβ8.1 CDR1 peptide does not generate increased antibody, but can cause diminution in titer (25). Consequently, it is noteworthy that retroviral infections in humans (5, 6, this paper) and mice (34) brings about major increases in autoantibodies to VβCDR1 epitopes.

The ACRS approach enables us to construct characteristic spectrotypes for populations of ASX individuals

TABLE 2

Synthetic Peptides Used in Study

(A) Synthetic Vβ CDR1 epitopes

Vβ2.1	CRSLDFQATTMFWYGQF
Vβ3.1	CVQDMDHEN MFWYRQD
Vβ4.1	CQVDSQVTM MFWYRQQ
Vβ5.1	CSPRSGDLS VYWYQQS
Vβ5.2	CSPKSGHDI VSWYQQA
Vβ6.1	CDPISGHTA LYWYRQS
Vβ6.5	CDPISEHNR LYWYRQT
Vβ7.1	CEQHMGHRA MYWYKQA
Vβ8.1	CKPISGHNS LFWYRQT
Vβ9.2	CEQNLGHDT MYWYKQD
Vβ10.1	CVPIKAHSY VYWYRKK
Vβ11.1	CSQTMGHDK MYWYQQD
Vβ12.1	CHQTENHRY MYWYRQD
Vβ13.1	CAQDMNHEY MYWYRQD
Vβ14.1	CSQNMNHEY MSWYRQD
Vβ15.1	CSQTKGHDR MYWYRQD
Vβ16.1	CDPISGHDH LYWYRRV
Vβ17.1	CEQNLNHDA MYWYRQD
Vβ19.1	CTPEKGHTF VYWYQQN
Vβ20.1	CTVEGTSNPNLYWYRQA
Vβ21.1	CDPISGHAT. LYWYRQI
Vβ22.1	CVPISGHSH VYWYRQL
Vβ23.1	CYPIPRHDT VYWYQQG
Vβ24.1	CSQTLNHNV MYWYQQK

(B) Synthetic Vβ FR3 epitopes

Vβ2.1	TVTSAHPEDSSFYICS
Vβ5.2	NLSSLELGDSALYFCA
Vβ6.3	KIQRTQQEDSAVYLCA
Vβ8.1	KIQPSEPRDSAVYFCA
Vβ12.1	TLESLPAPETSVYFCA
Vβ17.1	TVTSAQKNPIAFYLCA

Other peptides

pepJβ	ANYGYTFGSGTRLTVV
pepβ10	SANYGYTFGSGTRLTV
pepV3(HIV)	RIHIQRGPG•RAFYTTK

1:4000 for the anti-IgG and was incubated in the wells for 1 hr. After five washings with PBST, 0.03% 2,2'-azino-bis(3-ethyl-benzthioazoline-6-sulfonic acid) (Sigma Chemical Co.) in 0.1 *M* citrate buffer, pH 4.0, 0.01% hydrogen peroxide was added. The absorbances at 405 nm were measured in a Titertek Multiskan (Flow Laboratories), usually following 60 min of incubation at room temperature.

Titrations were carried out in duplicate beginning with the human serum diluted 1:50 with PBST followed by twofold serial dilutions. Titer was defined as the reciprocal dilution at which the ELISA absorbance had a value of 0.50 under standard conditions. It was obtained by numerical interpolation between the two dilution values bracketing the end point. A second quantitative approach was a one-step assay in which quadruplicate replicates were assayed at a serum dilution of 1:200 in PBST–gelatin. The third quantitative approach was use of a "modified sum absorbance" which utilized all of the data points in a titration by calculating the sum of the absorbencies at each dilution and correcting for nonspecificity by subtracting the sum of the values of the absorbencies of binding of the same serum or antibody preparation to uncoded PBST-blocked gelatin plates (24). This approach has the value of using all of the data and is useful for estimating small specific differences from background.

In order to determine the profile of autoantibody subsets within individual sera or antibody preparations, we constructed sets of synthetic peptides corresponding to CDR1 (Table 2A) and FR3 (of individual Vβ gene products). The assays were performed by two methods. The first was to determine the quantitative response to the individual "public idiotype." This was done by determining titer, absorbancy at a particular dilution or the "modified sum absorbance." The second application of ACRS was to determine the relative contributions of the individual subsets of autoantibodies. This was done in the case of the Vβ CDR1 homolog set by taking the value of the autoantibody binding to the Vβ8.1 peptide as 1.0 and normalizing the other values relative to it. We chose the Vβ8.1-CDR1 peptide as the referrant because it is the original one used in prior

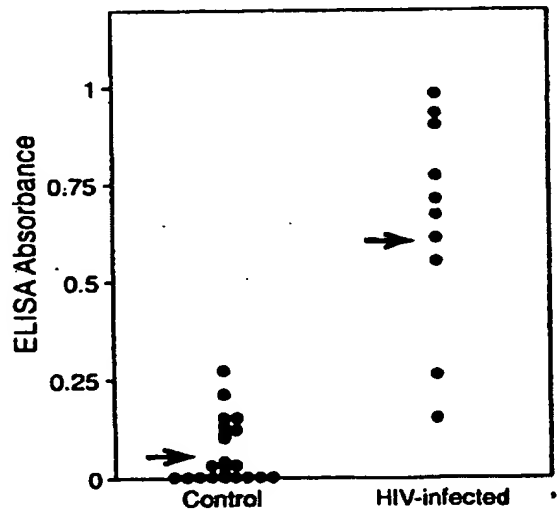

FIG. 1. Comparison of individual values for IgG autoantibody binding of 10 HIV+ patients and 22 healthy uninfected individuals to the scTCR. Individual sera were assayed in triplicate by ELISA at a dilution of 1/200. "Blank" values of binding of each serum to pig gelatin were subtracted from each value. The means of the two populations (normals, 0.06 ± 0.02; HIV+, 0.62 ± 0.09) differ significantly with $P < 0.01$. The means of the ELISA absorbance are indicated by arrows.

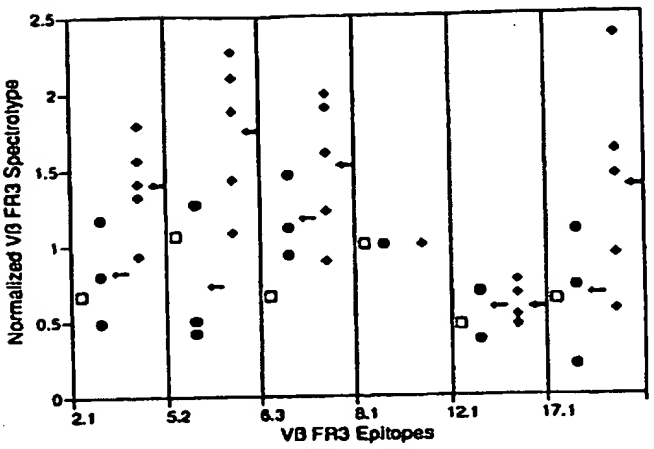

FIG. 12. Comparison of normal plasma pool with the individual coccidiomycosis and HIV-seropositive patients showing normalized spectrotypes to the Vβ FR3 epitopes. (□) Normal plasma pool; (●) coccidiomycosis; (♦) HIV-seropositive patient. In the two patient groups, the mean value is indicated by the —.

body reactivity is based upon binding to sets of homologous synthetic peptides corresponding to individual Vβ gene products. Recent studies by others have used quantitative immunoblotting to analyze the multiple V-region interactions among normal human IgG (35) and its repertoire for autologous protein tissue antigens (36). These approaches parallel the present one in demonstrating the capacity to recognize distinct profiles of polyclonal autoantibodies to Ig V-region determinants (35).

Although 8 of the 10 HIV-seropositive individuals followed were healthy during the full time course, one patient presented with ARC and progressed to AIDS by the last examination. The individual spectrotype comparison shows an overall increase in titer for most autoantibody specificities, but a simplification of the profile because of exceptional increases in binding of Vβ8.1, 10.1, and 22.1. Thus, certain autoantibody levels such as that to Vβ16.1 have increased in titer from 6393 to 7162 but decreased in relative contribution from 0.92 to 0.36. Moreover; although the titer to Vβ11.1 more than doubled from 2340 to 5144, the relative values declined from 0.34 to 0.26. By contrast, autoantibody titers to Vβ8.1, Vβ10.1, and Vβ22.1 epitopes each increased threefold and thus maintained their relative positions. A second patient was asymptomatic at presentation but developed AIDS at 2 years. The autoantibody CDR1 spectrotype of this individual, likewise, showed major reactivities for pepVβ8.1, 10.1, and 22.1 as well as relatively high binding to pepVβ2.1.

In general, autoantibodies to Vβ8.1 and 10.1 made the largest relative contributions, but substantial increases were observed in reactions to Vβ5.1, 9.1, 11:, 16.1, and 21.1 in some individuals. Furthermore, patient 2010 showed decreases in titer to Vβ8.1 and 10.1 coupled with marked increases to Vβ2.1, 3.1, 11.1, 12.1, 13.1, 16.1, and 17.1.

It will be necessary to obtain sera from individuals prior to infection and over time during the various stages of disease in order to obtain a complete understanding of the correlation of anti-TCR autoantibody production with retroviral infection and immunopathogenesis of HIV infection. The present study shows that generation of autoantibodies to TCR public idiotopes is a major consequence of infection with HIV, and that there is a selection for autoantibodies directed against subsets of Vβ gene products. Whether this is a consequence of autoimmunization to Vβ epitopes on T-cell subsets amplified by putative HIV superantigens (37–39) or of other mechanisms for generating autoantibodies to autologous TCR remains to be determined. Without preinfection baseline data for individuals, we cannot determine whether a population shift in specificities occurred consequent to the infections. However, the amount of antibody as indicated by titers increases substantially relative to those of uninfected individuals and internal studies of serial bleeds of infected individuals indicates that relative increases and decreases and population shifts can occur following infection.

ACKNOWLEDGMENTS

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UNMODIFIED OR MURINE MODEL

Immunomodulation by Immunopeptides and Autoantibodies in Aging, Autoimmunity, and Infection

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ABSTRACT: **The operation of the immune system is a complex orchestration of specific self and non-self–recognition capacities mediated by cells of the innate system acting in coordination with T and B lymphocytes in a series of processes modulated by cytokines. We provide evidence for a natural immunomodulatory system involving autoantibodies directed against a controlling segment of T cell receptor Vβ chains that downregulate production of stimulatory cytokines balanced by the peptides which in turn upregulate inflammatory activities mediated by TH1-type helper cells. TCR Vβ–derived peptides effective in retrovirally induced immunosupression could also reverse the effects of immunosenescence in aged mice by restoring the balance of TH1- and TH2-type immunity and the resistance of the animals to cardiac pathology caused by infection with coxsackievirus. An unexpected finding was an adaptive role of the T cells from peptide-treated mice in remodeling damaged hearts by increasing net collagen synthesis by cardiac fibroblasts.**

KEYWORDS: **T cell receptors; TH1; TH2; cytokines; retrovirus; coxsackievirus; immunosenescence; TCR Vβ; cardiopathology**

INTRODUCTION

Suppression of many aspects of mammalian immunity, particularly activities of TH1-type helper T cells, is a consequence of retroviral infection[1–4] or the normal processes of aging.[5–7] However, this activity can be elevated in T cell–mediated autodestructive diseases.[8,9] Here we address the antagonistic roles of spontaneously arising autoantibodies to T cell receptor (TCR) variable domains[10,11] and TCR Vβ–derived

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peptide epitopes[12,13] in maintaining the balance between TH1- and TH2-type immunities necessary for effective overall immunity.[14] We then illustrate the capacity of certain TCR VβCDR1 peptides to reverse negative effects of immunosenescence on the functional balance between TH1 and TH2 helper T cell subsets and on the restoration of resistance to cardiopathology following infection with coxsackievirus B in C57Bl/6 mice.[15]

NATURAL ANTIBODIES TO T CELL RECEPTOR VARIABLE DOMAINS

In studies designed to define and analyze the natural antibody repertoire common to vertebrate species, we investigated natural antibodies of man,[16–18] mouse,[19] and sharks.[20] Sharks are the lowest vertebrates to possess the genes essential for mounting the adaptive or combinatorial immune response[21] directed against a wide set of antigens including those involved in homeostasis, immunoregulation, and real-time response to infectious agents. Most notably, all three species possess spontaneously arising—that is, generated in the absence of purposeful immunization with the antigenic moieties—antibodies to shared idiotopes defined by the CDR1 segment of T cell receptor beta chain variable domains. The relative amounts and isotypes of these autoantibodies varied with physiological status, including aging[17,22] and pregnancy[23] as well as with allografts,[24] retroviral infections,[9,25,26] and autoimmune diseases—particularly rheumatoid arthritis and systemic lupus erythematosus.[11,16,17,22,27] On the basis of our findings in studies of rheumatoid arthritis and retroviral infections of humans and mice, we proposed that these antibodies directed against the VβCDR1 segments were part of an immunoregulatory process in which the body strove to downregulate T cells of an autodestructive character as defined by the overexpression of T cell subsets with varying particular Vβ gene products.[11] Studies by Jambeau and colleagues[28,29]—which reported that T cells of myasthenia gravis patients of the MHC class II DR3 haplotype showed a preferred restriction to Vβ5.1 and the levels of spontaneously arising autoantibodies to Vβ5.1 epitopes were inversely correlated with the severity of the disease—are also consistent with this hypothesis. Although these workers found autoantibodies to the CDR1 epitope, they found higher levels of autoantibodies to the CDR2 segment, and followed these in their investigations. Both the CDR1 and CDR2 segments are encoded by the Vβ gene and do not require rearrangement for their expression. In a rat autoimmunity model, Hashim and colleagues[30] reported that autoantibodies to the CDR2 segment of TCR Vβ8.2 arose spontaneously in animals injected with myelin basic protein in the generation of experimental allergic encephalomyelitis. Spontaneously arising T cell reactivity was also directed against the CDR2 peptide as well as to peptides corresponding to the third framework (FR3) and the CDR1 epitope.[31] It is noteworthy that naturally occuring human autoantibodies to VβCDr1 and FR3 epitopes were found in pooled normal IgG (IVIG) and in sera of patients with rheumatoid arthritis and systemic lupus erythematosus, although there was negligible reactivity to CDR2-associated peptides.[16]

Robey and colleagues determined that human monoclonals isolated from synovial and peripheral blood cells of rheumatoid arthritis patients reacted with the Vβ epitopes on the surface of D10-11 ovalbumin-specific T cells *in vitro*. This binding inhibited the production of the TH1-type cytokine IL2 following antigen stimulation

of the cells.[10] Additional evidence for the capacity of natural antibodies to TCR Vβ CDR1 epitopes to interact with and influence the function of antigen-specific T cells came from experiments using immune-affinity purified human IgG (from IVIG) and murine monoclonal antibodies derived from splenic B cells of retrovirally infected mice. Cytotoxic murine T cells specific for the breast cancer–derived tumor EMT6 expressed predominantly Vβ1 or Vβ8 receptors.[32] Human natural antibodies specific for CDR1 peptide–defined epitopes of these Vβ gene products were isolated by immune affinity chromatography from IVIG and found to inhibit killing of the tumor cells *in vitro*. Virtually complete inhibition occurred when both purified antibody subsets were added simultaneously.[11] The murine monoclonal autoantibodies were able to synergize selectively with T cell superantigens,[33] thereby indicating binding to the combining site region of the TCR and modulating the signal given by the superantigen. The production and characterization of the monoclonal anti-TCRs have been described in detail elsewhere.[34,35] These results indicate that a likely function of these spontaneously arising anti TCRVβ antibodies is to downregulate TH1 type immunity.

IMMUNOMODULATION BY TCR Vβ–DERIVED PEPTIDES

We carried out experiments designed to determine whether the peptides themselves had any effect on the diminished immune system of C57Bl/6 mice infected with the LP-BM5 strain of murine leukemia virus. This infection produces a condition termed mouse AIDS (MAIDS), characterized by suppression of TH1-type immunity, enhancement of TH2 immunity, and an overall pattern of acquired immunodeficiency.[12] It was found (TABLE 1) that peptides corresponding to the CDR1 segment of human Vβ8.1 (now termed BV12s3) and Vβ5.2 reversed many of these negative effects induced by the retroviral infection, particularly restoring the balance between TH1 and TH2 activity, by upregulating TH1-type cytokines, such as IL-2 and INFγ, and downregulating TH2 cytokines, including IL4 and IL6.[12,13,36–38] FIGURE 1 shows the sequences of the human peptides, their closest murine homologues, and a peptide derived from the CDR1 segment of the human lambda light chain MCG that served as a negative control in functional studies. Furthermore, the peptide-treated immunodeficient mice regained the capacity to resist infection by protozoan parasites such as *Cryptosporidium pavum*[38] and to regain resistance to the generation of cardiac pathology induced by secondary infection with coxsackievirus B3.[39] Healthy C57Bl/6 mice are not susceptible to infection by *C. parvum* or to the development of cardiac pathology following infection with coxsackie virus. How-

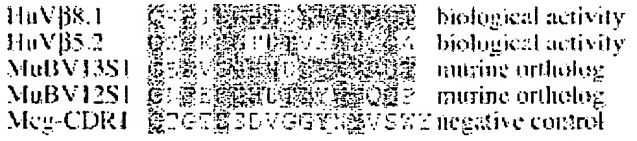

FIGURE 1. Comparative alignment of human and murine Vβ CDR1 segments illustrating homology between the two sets. The CDR1 segment of the human Vλ5 light chain is also shown. It was frequently used as a negative control in functional studies of peptide administration to MAIDS or aged mice.

TABLE 1. Summary of effects of treatment with pep Vβ8.1 CDR1 on immunological responses of C57Bl/6 mice infected with LP-BM5 strain of murine retrovirus (MAIDS)

Parameter	Murine AIDS	Effect of pep Vβ8.1
T cell mitogenesis	↓	↑
B cell miogenesis	↓	↑
NK cell activity	↓	↑
IL-2	↓	↑
INF-γ	↓	↑
INF-α	↑	↓
IL-4	↑	↓
IL-5	↑	↓
IL-6	↑	↓
IL-10	↑	↓
Ig synthesis	↑	↓
Splenomegaly	↑	↓
Resistance to parasites	↓	↑

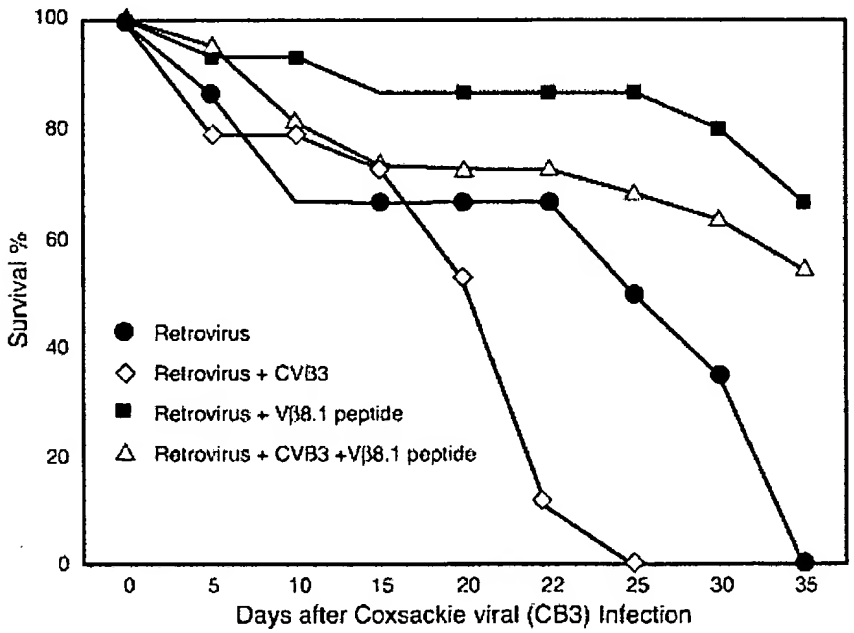

FIGURE 2. Survival curves for retrovirus-infected mice (LP-BM5) co-infected with coxsackie virus CVB3 (at 3 months following LP-BM5 infection) compared with animals given retrovirus alone, retrovirus plus TCR Vβ8.1 peptide, and retrovirus plus CVB3 plus Vβ8.1 peptide. Vβ8.1 peptide was administered as two injections within two weeks of the original retrovirus injection. Twenty mice were used per group. In addition, a control group of 10 non–retrovirally infected animals were followed during the course of the experiment. All survived the complete course of the trial (data not shown).

ever, immunosuppression either by retroviral infection or as a consequence of normal aging renders them susceptible to both deleterious consequences.

FIGURE 2 illustrates the enhancing effect of the Vβ8.1 peptide on survival of mice infected with the LP-BM5 retrovirus alone as well as those infected with coxsackievirus B3 in addition. Mice were infected with LP-BM5. The peptide-treated groups were given two intraperitoneal injections of peptide (200 μg per injection) within two weeks following the experimental infection. Sixteen animals were used per group. At three months following the LP-BM5 injection, two groups were infected with coxsackievirus. All of the mice given retrovirus and coxsackievirus, but not TCR Vβ8.1 peptide, died of cardiac failure by day 25. Unprotected animals given retrovirus alone survived approximately an additional 10 days. By contrast, approximately 60% of LP-BM5–infected mice given coxsackievirus plus TCR peptide were alive at day 35. More than 70% of peptide-treated LP-BM5–infected mice that were not given coxsackievirus survived until the end of the experiment at 35 days following initiation of the coxsackievirus infection. Coxsackievirus B–induced cardiopathology in aged mice will be considered below.

Analysis of the effects of the anti–TCR Vβ autoantibodies and the peptides themselves on the immune system, led us to propose the immunomodulatory scheme illustrated in FIGURE 3. Peptides generated from a control segment of endogenous T cell receptors upregulate TH1 immunity, and this is balanced by spontaneously arising autoantibodies to that segment. We consider the CDR1 loop, particularly of human Vβ8.1 (BV12s3), to be a "control segment" because it is the least variable of

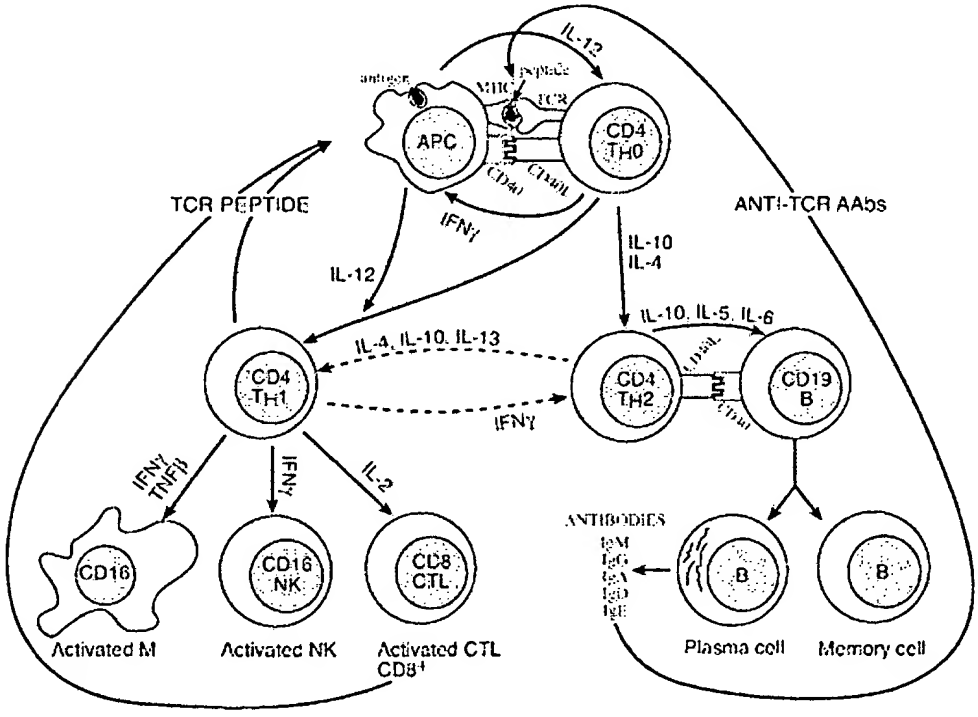

FIGURE 3. Scheme for the modulation of the immune response by TCR-derived peptides that upregulate TH1-type immunity and spontaneously arising anti-TCR Vβ autoantibodies that suppress this activity.

TCR Vβ hypervariable segments and shows 50% or more identity in comparison among the human Vβ chains. We were fortunate to be able to carry out functional studies *in vitro* and *in vivo* with mice using human peptides and antibodies because of the high degree of homology between T cell receptor Vβ segments of man and mouse.

APPLICATION TO IMMUNOSENESCENCE

Analysis of natural autoantibodies (NAABs) of humans ranging from approximately 20 to 90 years of age gave results consistent with general expectations that some autoantibodies would increase with age.[40,41] We found in particular that anti-TCR autoantibodies of the IgM isotype tended to decline with age, a result particularly noticeable after age 50, whereas autoantibodies of comparable specificity in the IgG isotype tended to increase in later years.[17] This phenomenon is illustrated in FIGURE 4 for spontaneously arising NAABs of healthy young (Y) individuals 20–40 years of age as compared with healthy elderly (E) individuals more than 70 years of age. This summary plot gives the frequency of positive sera per group to facilitate comparison. Statistical analysis of the original quantitative data using the Wilcoxon test supported the decrease in IgM NAABs with age (e.g., $P < 0.001$ for activity against pepB3) and the increase in IgG activity with aging (e.g., $P < 0.02$ for the pepVβ2.1 comparison). The fact that both groups had active immune systems is shown by their IgG antibodies to the common environmental antigen ovalbumin. Parallel results to those found to CDR1 peptides were also observed (not shown) with certain peptide epitopes corresponding to band 3 peptides associated[17] with the senescent cell antigen.[42]

The findings with the murine retrovirally immunosuppressed model suggested that comparable results may occur in the situation where immunosuppression is a natural consequence of aging. The thymus involutes substantially following puberty in humans.[43] In mice, the total number of thymocytes drops by more than 80% by 16 months of age.[44] This decline is associated with diminished lymphocyte prolifer-

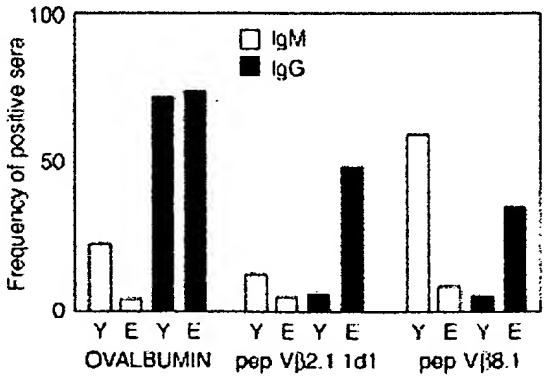

FIGURE 4. Comparison of IgM and IgG responses of healthy young (Y) and elderly (E) individuals to ovalbumin and to synthetic TCR CDR1 region peptides Vβ2.1 andVβ8.1. The data are given as frequency of positive set per group. The young group consisted of 28 individuals and the elderly group of 29. (Based on data of Marchalonis and colleagues.[17])

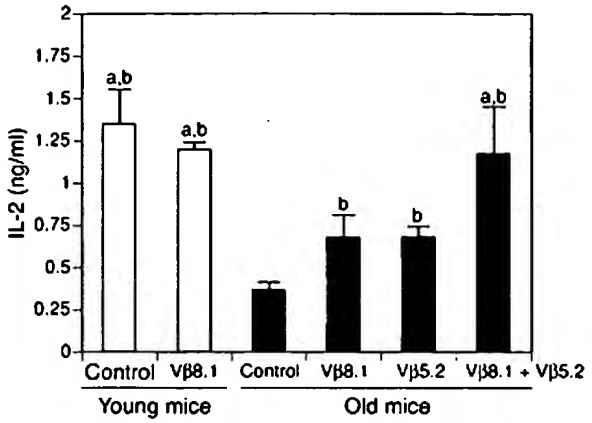

FIGURE 5. Effect of TCR peptide injection on IL-2 production by concanavalin A–timulated splenocytes *in vitro* from young (4 weeks of age) and old (16 weeks of age) C57/16 mice. Assays were performed in triplicate with data presented as mean ± standard devition ($N = 6$). The letters indicate significant differences at $P < 0.05$: (**a**) compared with the ld-mice group (with the exception of the group given both pep Vβ8.1 and pep Vβ5.2) and **b**) compared with the control old-mouse group. (Based on the work of Liang and olleagues.[14])

ative capacity, decreased production of TH1 cytokines, and increased level of TH2 cytokines as well as increased levels of certain autoantibodies.[7,45] In addition, aged C57 BL/6 mice (16 months or older) resemble retrovirally immunosuppressed animals in becoming susceptibile to cardiopathology following infection with coxsackie virus B3.[15] FIGURE 5 illustrates a comparison in IL-2 production by concanavalin-A–stimulated splenocytes obtained from 4-week-old C57Bl/6 mice compared with comparable cells from animals 16 months of age. *In vitro* production of IL-2 by cells in the older animals was significantly ($P< 0.05$) lower than that produced by splenocytes from the young animals. Injection of the mice with Vβ 8.1 and Vβ 5.2 TCR–derived peptides, either individually, or in combination substantially increased the production of IL-2 by the mitogen-stimulated splenocytes of the older mice. FIGURE 6 illustrates the opposite situation, where the TH-2-type cytokine IL-4 is substantially increased in older animals relative to the young mice. However, this level is significantly decreased by the TCR Vβ–derived peptides. Control peptides, such as the homologue prepared from the CDR1 segment of human λ myeloma protein MCG, had no effect, and results were indistinguishable from those obtained when the animals were merely given saline instead of peptide solutions.

Neither young nor mature healthy C57BL\6 mice (3–10 months old) develop cardiopathology when challenged with coxsackievirus B3 (CVB3). To determine whether treatment with TCR Vβ 8.1 peptide could protect aged mice from the development of cardiopathology following CVB3 virus infection, we infected mice at 18 months of age with CVB3, sacrificing them at 12 days following infection to assess the extent of mononuclear cell infiltration and necrosis by histopathological examination of fixed, eosin-stained heart tissue. The results of this analysis are given in FIGURE 7. The severity of histopathology is scored as 1+, <10% heart tissue affected; 2+, moderate, with 10–25% tissue affected; and 3+, greater than 25% of the tissue showing evidence of necrosis and infiltration. Aged CVB3-infected mice

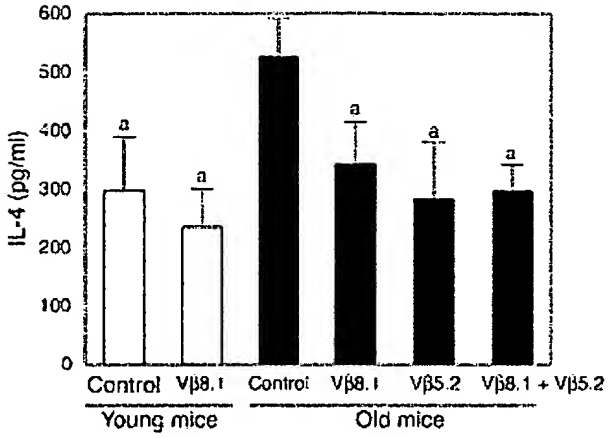

FIGURE 6. Effect of TCR peptide injection on IL-4 production *in vitro* by concanavalin A–stimulated splenocytes of young and old C57/ B16 mice. Values are mean ± standard deviation of triplicate assays on cells from 6 animals. (a) Significant difference at $P < 0.05$ by comparison with the old control group. (Based upon work by Liang and colleagues.[14])

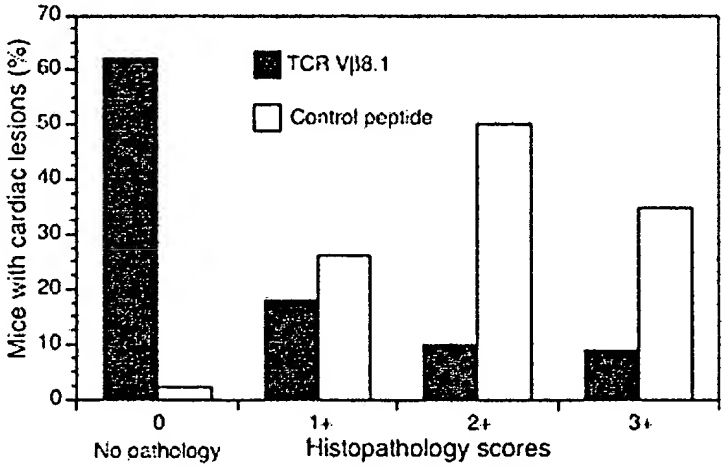

FIGURE 7. Histopathologic scores of aged C57/B16 mice infected with coxsackievirus B3 and treated with TCR peptide Vβ8.1 or peptide control. $N = 12$ samples per group. Pathologic score: 0, no lesions; 1+, mild multifocal nonsuppurative epicarditis to mild multifocal nonsuppurative myocarditis; 2+, mild focal to multifocal nonsuppurative myocarditis with myocardiocyte degeneration and necrosis; 3+, moderate focal to multifocal nonsuppurative myocarditis with myocardiocyte degeneration and necrosis; 4+, severe multifocal nonsuppurative myocarditis with myocardiocyte degeneration and necrosis. Mild damage is considered as <10% of heart tissue affected, moderate as 10–25%, and severe as >25%. (Based upon data of Sepulveda and colleagues.[15])

TABLE 2. Summary of effects of TCR peptide administration on parameters showing immunosenescence in aged mice

Cytokine or test	Old	Old + TCR peptide
B cell proliferation	↓	↑
T cell proliferation	↓	↑
IL-2	↓	↑
TNF-α	↑	↓
IL-6	↑	↓
IL-4	↑	↓

treated with the control peptide showed cardiopathology scores of 1+ (25%), 2+ (50%), and 3+ (25%). However, when identically infected mice were treated with TCR Vβ 8.1 peptide, 62% of the group showed no histopathology, with 18% showing 1+ scores, 10% showing 2+ scores, and only 9% showing 3+ scores. As would be expected from the enhanced TH1 activity of the aged mice following administration of Vβ 8.1 peptide, the burden of viruses infecting the heart, as judged by cardiac viral titer, was significantly diminished ($P<0.05$), with a decrease of approximately 80%.

The results with the aged mice documented the relative lack of histopathology in hearts of coxsackie-infected mice given the TCR Vβ peptide. Examples of phenomena correlated with immunosenescence and the effects of administration of TCR Vβ peptides are summarized in TABLE 2.

CONTRIBUTION OF T CELLS TO REMODELING HEARTS DAMAGED BY CVB INFECTION

Additional studies were carried out with the mice that were retrovirally immunosuppressed and given the TCR Vβ 8.1 peptide prior to CVB3 infection. They showed a restoration of cardiac function and collagen remodeling of the cardiac tissue.[46] Left ventricular stiffness was significantly increased in the treated mice, whereas it was decreased in those given retrovirus in the absence of TCR-derived peptide. Furthermore, the latter group also showed significantly increased end-diastolic and end-systolic volumes indicative of decreased functional pumping capacity. The results with the TCR peptide–treated animals indicative of a collagen remodeling within the heart were unexpected and surprising. In order to address possible explanations for the phenomomen, we performed experiments to determine whether splenic lymphocytes taken from uninfected animals, LP-BM5–infected animals, or LP-BM5–infected animals given a mixture of Vβ 8.1 and 5.2 peptides could interact *in vitro* with isolated cardiac fibroblasts to produce the collagen needed to remodel the coxsackievirus-damaged heart tissue. FIGURE 8 shows the effects of co-culturing these lymphocytes *in vitro* with cardiac fibroblasts. Lymphocytes from 3 mice in each group were pooled and added to the cardiac fibroblast cultures at a concentration of 10^5/mL. After 48 h of co-incubation the lymphocytes were removed and the cardiac fibroblast

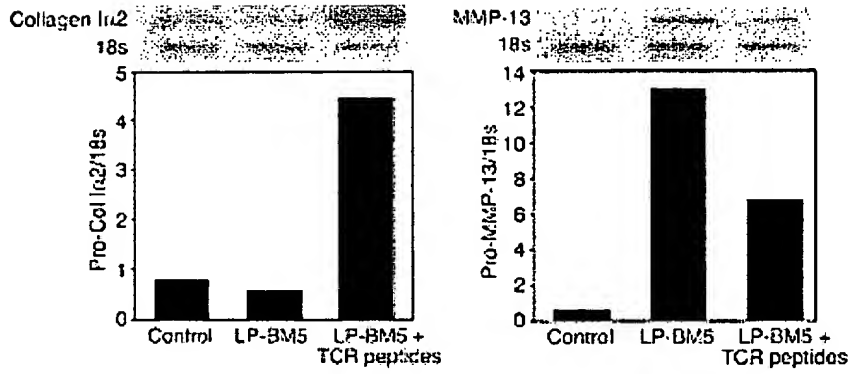

FIGURE 8. Modulation by lymphocytes of collagen (Iα2) and matrix metalloproteinase (MMP-13) synthesis by cardiac fibroblasts following *in vitro* co-culture. Splenic lympocytes were harvested from the control, LP-BM5 only, and combined LP-BM5 plus TCR peptide mice 12 weeks after treatment and co-cultured with naïve primary cardiac fibroblasts. Lymphocytes from three mice in each group were pooled and added to the cardiac fibroblast cultures at a concentration of 5×10^5/mL. After 48 h of co-incubation, the lymphocytes were removed and the cardiac fibroblast RNA analyzed for pro-collagen Iα2 and pro-MMP-13 with RT-PCR. The graphed data are the ratio of the candidate gene versus 18s RNA.

mRNA analyzed for pro-collagen 1 α2 and pro-matrix metalloproteinases (MMP)-13 using RT-polymerase chain reaction. The graphed data are the ratio of the candidate gene versus 18s RNA. LP-BM5 treatment alone caused a 30% decrease in synthesis of pro-collagen 1α2 and a 13-fold increase in pro-MMP-13 cardiac fibroblast gene expression. However, the addition of the TCR peptides to the LP-BM 5 groups resulted in a sixfold increase of pro-collagen and a twofold decrease in pro-MMP-13 gene expression. Thus, the effect of the treatment with the T cell peptides is to increase net collagen synthesis and decrease the level of matrix metalloproteinase that would degrade the collagen. Cross-linking of the collagen to impart stiffness was also increased. These results were confirmed for collagen by Western blot analysis showing an actual increase in the collagen as antigenic protein. The MMP results were supported by assay of enzymatic activity. Supernatants isolated from *in vitro* cultured lymphocytes of TCR peptide–treated animals likewise showed increased production of collagen and decreased expression of MMP activity. Experiments are in progress to determine what specific T cell–produced factors or cytokines are involved.

CONCLUSIONS AND DISCUSSION

The data presented here support the conclusion that the broad decline in TH1 function in aging can be reversed, at lease partially, by immunomodulatory peptides derived from a "control region," the CDR1 segment, of TCRVβ. This process parallels the immunodulatory effects of the TCR peptides in murine retroviral infection, where they restore the balance between TH1 and TH2 activities. We stress that this is a restoration of balance required for normal immune function because we have not observed overshoots in levels of TH1 cytokines relative to those produced by healthy immunologically normal C57BL/6 mice. The increases we observed in the immuno-

logically deficient animals treated with appropriate TCR peptides approached the normal levels asymptotically. This strain of mouse generally gives a strong TH1-type response to microbial infection and it is necessary to use animals rendered immunodeficient by either retroviral infection or advanced age to demonstrate the restorative effects of the TCR Vβ–derived immunomodulatory peptides. The peptides found to be effective are derived from human Vβ sequence, but are extremely similar to homologues occurring in orthologous murine Vβ sequences. We have not observed any adverse effects following administration of high peptide doses to mice, and a Phase I safety trial carried out by Allergene, Inc. indicates that the Vβ8.1 peptide should be safe for human use at the doses tested. Efficacy trials have not yet been performed for human subjects (personal communication, W. Friedman, Allergene).

Spontaneously arising autoantibodies to the CDR1 segment downregulate TH1 activity, suggesting that a novel immunoregulatory mechanism acts in autoimmunity and normal immunization. Although we have focused on the VβCDR1 peptide in our studies, other workers have found spontaneously arising autoantibodies to the VβCDR2 segment in human autoimmune disease[28] and in rodent experimental autoimmunity.[30] The results of those studies are consistent with the model proposed here. This scheme resembles somewhat the idiotypic networks originally proposed by Jerne[47] for regulation of antibody production. However, it is quite distinct because the typical idiotypic models focus on the CDR3 segment formed by recombination of V, D, and J gene segments and is further diversified by junctional processes to form highly unique or individual markers. By contrast, the CDR1 segment used here is completely specified by the germline Vβ gene and shows a substantial degree of sequence identity (50% or greater) among all human Vβ genes reflected in antigenic crossreactions shown by monoclonal anti-Vβ autoantibodies.[35]

We found an unexpected role of TCR peptide–stimulated T cells in remodeling defective heart cells that had been infected with coxsackievirus. Net collagen synthesis was increased via downregulation of matrix metalloproteinase.[46] Experiments in which lymphocytes harvested from TCR peptide–treated mice were co-cultured *in vitro* with naïve primary cardiac fibroblasts showed that the lymphocytes exerted a direct control on fibroblast gene expression of pro-collagen, pro-MMP, and MMP enzymatic activity. These results raise the possibility that the helper T cell phenotype can differentially affect diastolic function and that modulation of TH activity could promote adaptive remodeling in heart failure and post-myocardial infarction. Traditionally, endocrine and mechanical factors are thought to be the major regulatory factors in these processes, but the immune system through TH polarization and subsequent generation of cytokines may play a substantive role as well.

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[54] **T-CELL RECEPTOR PEPTIDES AND METHODS FOR PREVENTING THE PROGRESSION TO AIDS IN AN ANIMAL MODEL**

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[21] Appl. No.: **08/696,049**

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[51] **Int. Cl.**[6] **A61K 39/00**; A61K 45/05; C07K 14/725

[52] **U.S. Cl.** **424/185.1**; 424/278.1; 530/300; 530/395

[58] **Field of Search** 530/300, 350, 530/395, 868; 424/185.1, 207.1, 184.1, 278.1

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(List continued on next page.)

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[57] **ABSTRACT**

A novel peptide from the T-cell receptor is shown to be effective in preventing the progression to AIDS in an animal model. Methods for delaying the progression to AIDS and restoring normal immunological responses in an animal model following infection are shown and comprise administering through various systemic routes T-cell receptor peptide Vβ CDR1 to restore normal levels of Th1 cytokines interleukin 2 and interferon-γ, which are suppressed following infection, and those of Th2 derived cytokines interleukin 5, interleukin 6, interleukin 10, and immunoglobulin G, which are stimulated following infection.

6 Claims, No Drawings

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T-CELL RECEPTOR PEPTIDES AND METHODS FOR PREVENTING THE PROGRESSION TO AIDS IN AN ANIMAL MODEL

The U.S. Government has paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant Nos. AA-08037 and CA-42049 awarded by the National Institute of Health.

FIELD OF THE INVENTION

The present invention relates generally to a novel treatment for use in preventing the progression to AIDS in an animal model. Unlike most present efforts now being made to treat HIV and AIDS with antiviral drugs, and the push to develop new antiviral drugs that are effective for treating AIDS, the present invention relates to the novel use of a peptide derived from human T-cell receptors.

BACKGROUND OF THE INVENTION

Acquired immune deficiency syndrome (AIDS) is a disease of retroviral etiology, characterized by immune dysfunction, opportunistic infections, and eventually death. Murine acquired immune deficiency syndrome (MAIDS), induced by infection with the murine LP-BM5 leukemia retrovirus (MuLV) mixture, causes a progressive and profound immunodeficiency. It is strikingly similar to human AIDS, with splenomegaly, lymphadenopathy, and hypergammaglobulinemia in the early stage of retrovirus infection, progressive defects in T and B cell function, and reduction of host resistance to pathogens and neoplasia. These similarities exist even though human immunodeficiency virus (HIV), a lentivirus, and the retrovirus causing murine AIDS, MuLV, a C-type retrovirus, represent different types of retroviruses (1).

The immunopathogenic mechanisms underlying HI V infection and disease are not unidimensional; they are extremely complex (2). Preferential expansion, deletion, and activation of some $CD4^+$ $\alpha\beta$ T cells induced by retroviral super or chronic antigen (Ag) exposure in human and murine AIDS may be important immunopathogenic mechanisms (3–5). Selective Ag activation of $CD4^+$ $\alpha\beta$ T cells may lead to polyclonal stimulation of T and B cells at early stages, with subsequent aberrant cytokine production $CD4^+$ T cell depletion. Eventually, these abnormalities lead to profound immunosuppression of cell-mediated immunity and immunodeficiency (2).

The aberrant cytokine production due to retrovirus infection, caused by a switch from T helper 1 (Th1) response to T helper 2 (Th2) response, promotes the progression to AIDS (6). In HIV^+/AIDS patients and MuLV-infected mice, T cell proliferation and Th1 cytokine (interleukin-2 (IL-2) and interferon-γ) production decline, while Th2 cytokine (IL-4, IL-5, IL-6, and IL-10), and Ig production increase (7–10). The Th1 to Th2 conversion may determine the fatal outcome of the disease as part of the mechanism producing severe immunodeficiency and loss of disease resistance during the progression to AIDS. When IL-4-deficient mice (IL-4 gene knockout) that are defective in Th2 cytokine responses are infected with LP-BM5 retrovirus, there is no lethality, and the development of T cell abnormalities associated with murine retrovirus infection is delayed (11). Administration of anti-IL-4 monoclonal antibody (Mab) to LP-BM5 retrovirus-infected mice or restoring the Th1 cytokine, IFN, by injection also normalizes the imbalance of

Th1 and Th2 responses induced by retrovirus infection, prevents retrovirus-induced suppression of immune responses, and alleviates the typical murine AIDS symptoms: splenomegaly and hypergammaglobulinemia (12).

Autoantibodies (AAb) binding a peptide determinant corresponding to the first complementarity determining region (CDR1) of the T-cell receptor (TCR) Vβ domain were elevated early in murine retrovirus infection (13). Elevation of the levels of these AAbs is an early event following retroviral infection that corresponds in part to the general polyclonal activation of B cells with selectivity for particular Vβ sequences that occurs later. The production of high levels of anti-TCR AAb early in this disease with continued production of some AAbs suggests that they might be involved in retrovirus immunopathogenesis. The AAb directed against CDR1 determinants can be considered natural Ab against public or regulatory idiotypes (Id) (14), since this region is the least variable of the CDR and is completely specified by the Vβ gene sequence.

Preferential expansion of some TCR $\alpha\beta$ $CD4^+$ T cells induced by retroviral superantigens in both human and murine retrovirus infection is an important immunopathogenic mechanism (2, 5). Selective expansion/deletion of some TCR $\alpha\beta$ $CD4^+$ T cells may lead to polyclonal activation of T and B cells at an early stage, and subsequent aberrant cytokine production. Eventually these abnormalities lead to profound immunodeficiency with immunosuppression of cell-mediated immunity.

SUMMARY OF THE INVENTION

The present invention provides a T-cell receptor (TCR) peptide which, when administered to a host infected with an immunodeficiency-type retrovirus, is effective in preventing the progression to AIDS. The present invention also provides methods for preventing the progression to AIDS in an infected host, comprising administering TCR Vβ CDR1 peptide to an infected host by various systemic routes, thus arresting the development of the immune dysfunction and cytokine dysregulation which allow retrovirus infections to weaken the host facilitating life-threatening pathogens.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present invention, administering a peptide corresponding to the first complementarity determining region of the T-cell receptor Vβ domain (TCR Vβ CDR1) and having the sequence Cys Lys Pro Ile Ser Gly His Asn Ser Leu Phe Trp Tyr Arg Gln Thr (SEQ ID NO:1) to a host infected with an immunodeficiency-type retrovirus prevents retrovirus-induced suppression of immune responses and normalizes cytokine production. The TCR Vβ CDR1 peptide, a synthetic molecule, was obtained under contract from various suppliers, including the Department of Biochemistry and Molecular Biology at the Medical University of South Carolina, Charleston, and the University of Arizona Biotechnology Center, Tucson. The peptide was synthesized using Applied Biosystems Peptide Synthesizers, and the purity was determined by amino acid composition and sequence analysis. When necessary, peptides were purified by high performance liquid chromatography (HPLC) under reverse phase conditions. The average purity of the peptide prior to purification by HPLC was 90%.

The present invention provides methods of delaying the onset of AIDS, comprising administering by various systemic routes TCR Vβ CDR1 peptide to a host infected with an immunodeficiency-type retrovirus in order to restore

normal levels of Th1 cytokines, such as interleukin 2 and interferon-γ, and Th2 derived cytokines, such as interleukin 5, interleukin 6, interleukin 10, and immunoglobulin G. The present invention also provides methods for reversing the deleterious effects of infection with an immunodeficiency-type retrovirus, comprising administering TCR Vβ CDR1 peptide to a host infected with said immunodeficiency-type retrovirus, to restore normal levels of Th1 cytokines, such as interleukin 2 and interferon-γ, and Th2 derived cytokines, such as interleukin 5, interleukin 6, interleukin 10, and immunoglobulin G.

In addition, the present invention provides methods for modulating the immune response in a mammal infected with an immunodeficiency-type retrovirus, comprising administering by various systemic routes an amount of TCR Vβ CDR1 peptide sufficient to restore normal levels of Th1 cytokines and Th2 derived cytokines. Methods of suppressing progression to immune dysfunction and cytokine dysregulation caused by HIV infection, comprising administering TCR Vβ CDR1 peptide in an amount sufficient to restore normal levels of Th1 cytokines and Th2 derived cytokines, are also shown.

Moreover, methods of preventing immunosuppression and cytokine dysregulation induced by infection with an immunodeficiency-type retrovirus, comprising administering systemically to an infected host TCR Vβ CDR1 peptide in an amount sufficient to restore normal immunological functions, are shown. Methods are also shown for altering the immune system response of a host infected with an immunodeficiency-type retrovirus, comprising artificially introducing a TCR Vβ CDR1 peptide into the bloodstream or immune system by injection so as to artificially stimulate the immune system to restore normal levels of Th1 cytokines and Th2 derived cytokines.

Systemic routes that may be used for administering the TCR Vβ CDR1 peptide in all embodiments of the present invention include intravenous injection, intraperitoneal injection, oral administration, subcutaneous administration, intramuscular administration, and administration by autologous dendritic cells. Oral administration usually leads to tolerance, whereas the other systemic routes lead to distinct types of immunity that may be particularly useful in certain infections or disease states. The peptide may be administered with or without adjuvant such as alum, Freunds complete or incomplete adjuvants, poly (AU) or RIBI adjuvant, or coupled to a carrier such as albumin, ovalbumin, or other native or engineered proteins.

Typically, doses of 5 mg/kg of body weight to 25 mg/kg of body weight of TCR Vβ CDR1 peptide in saline are administered in divided doses following infection. Preferably, doses of approximately 10 mg/kg of body weight to 25 mg/kg of body weight of TCR Vβ CDR1 peptide in saline are administered in divided doses. Most preferably, doses of 10 mg/kg of body weight are administered. Multiple doses administered approximately once per month increase the efficacy of the TCR Vβ CDR1 peptide therapy. Dosage amounts, however, may vary depending on the route of administration and depending on whether the TCR Vβ CDR1 peptide is administered with or without adjuvant.

The theory underlying the TCR Vβ CDR1 peptide administration approach is that it should be beneficial in any situation, whether it be infectious, autoimmune, or environmental, in which autoantibodies against the family of TCR Vβ CDR1 peptides are generated. This includes infection with C-type retroviruses MULV/MAIDS), lentiviruses (HIV including HIV-1, HIV-2, and HIV-3, simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV)), infection with non-viral pathogens (e.g. coccidiomycosis), and contact with environmental agents such as oils and adjuvants that amplify various features of the immune system. In all embodiments of the present invention, infections by immunodeficiency-type retroviruses that may be challenged by administering TCR Vβ CDR1 peptide include C-type retroviruses and lentiviruses. The changes in retrovirus-induced immune dysfunction and cytokine production in the infected host following administration of the TCR Vβ CDR1 peptide occur simultaneously with restoration of tissue vitamin E, a mild immunostimulant and reduced lipid peroxidation in tissues, which decreases the oxidative stress caused by free radical products, i.e., lipid fluorescence and diene conjugates.

Most antigens (Ag) are recognized through their interaction with the variable (V) portions of the TCR α- and β-chains. However, T cells recognize another category of ligands, the superantigens, on the basis of the expressed Vβ region alone, independently from the other variable TCR segments. The progression of $CD4^+$ T cell expansion/depletion requires stimulation of T cell clones or subgroups with retroviral chronic or superantigen exposure, resulting over time in excessive activation followed by energy of $CD4^+$ T cells bearing Ag-selected Vβ. AAb against peptide-defined epitopes of TCR that were used to select the peptide for administration studies here were also found in high levels in HIV^+ patients (12). This overproduction may result from a failure in regulation.

Two potential mechanisms may be responsible for prevention of immune dysfunction during immunodeficiency-type retrovirus infection and concomitant TCR Vβ CDR1 peptide administration: (1) immunization against specific regulatory determinants on the products of individual Vβ genes, and (2) the possibility that peptides corresponding to the CDR1 and Fr2 segment of the Vβ-chain could interact with MHC molecules necessary for the presentation of peptide Ag, altering this process (24). A general mechanism of this nature is required because the effective peptide corresponds to only one of a possible set of >30 peptides, and it is unlikely that any single one would have an overall regulating effect based upon its individual ID specificity. However, immunological cross-reactivity between the TCR Vβ CDR1 peptide and other CDR1 sequences is expected because 51% of 51 major human TCR Vβ gene family sequences show at least 50% identity to the TCR Vβ CDR1 peptide. Both affinity purified naturally occurring human autoantibodies and monoclonal hybridomas expressing naturally occurring human and murine autoantibodies to the TCR Vβ CDR1 peptide can cross-react with between 2 and 7 other CDR1 peptides out of a test set of 24 individual human Vβ gene products, thus indicating cross-reactivity. The full 16 residue length is required for binding of the autoantibodies (Marchalonis, unpublished) as well as for the correction of the immunological defects induced by retroviral infection (34).

AAb to TCR Vβ induced by administration of TCR Vβ CDR1 peptide may slow the selective deletion/expansion of some Vβ T cells by cytolysis or other inhibitory mechanisms, including obstructed binding of the Ag to TCR Vβ-chains. The increased production of AAb to the TCR Vβ CDR1 peptide alone during AIDS may not be sufficient to alter the TCR Vβ expression profiles induced by retroviral super or chronic Ag exposure. Retrovirus infection induced selective induction of high levels of AAb against the TCR Vβ CDR1 peptide, presumably due to increased TCR Vβ T cell expression. The prevention of retrovirus-induced immu-

nosuppression and cytokine dysregulation by TCR Vβ CDR1 peptide administration may be due to a difference in the affinities of the different Vβ for the superantigen after specific AAb interference.

Patients infected with HIV display a progressive loss of $CD4^+$ Th cell function, often taking years before cell numbers and other cell functions are depressed sufficiently to produce AIDS (37). The loss of resistance to HIV infection and/or progression to AIDS may be dependent on a switch from Th1- to Th2-subset dominated responses (9). Progression to AIDS is characterized by decrease in Th1-cytokine (IL-2 and IFN-γ) production concomitant with an increase in Th2-cytokine (IL-6 and IL-10) production. Th1 and Th2 cytokine profiles in retrovirus-infected mice are in accordance with this hypothesis (14, 15). Similarly, progression to severe pathology due to murine retrovirus infection is characterized by loss of Th1 cytokine (IL-2 and IFN-γ) production concomitant with increases in Th2 cytokine (EL-5 and IL-10) production (9, 14, 15). Th1 and Th2 cytokine profiles in murine retrovirus infection are in accordance with this hypotheses.

The presence of a dose-response relationship as well as significant immune preservation in the presence of low doses of peptide in combination with two adjuvants further suggests an immunoregulatory mechanism. TCR Vβ CDR1 peptide administration before or early in the infection could slow activation of T cells, as we found a smaller increase in $IL\text{-}2R^+$ activated cells in immunized infected animals than in the infected unimmunized mice. Thus, during TCR Vβ CDR1 peptide administration, fewer cells may be activated to become Th2 or permitted to remain as immature Th0 cells with their high production of IL-4 and IL-10. This would preserve, but not restore, normal function in most bystander cells that were close enough to be affected by cytokines produced by T cell clones stimulated by super or chronic retroviral Ag exposure without the increased IL-4 production by Th2 cells. In this situation, more cells would remain as Th1 cells, producing IFN-γ and IL-2 during retrovirus Ag exposure. They would also suppress Th2 cytokine production in neighboring cells.

Recent studies have demonstrated in C57B1/6 mice that the stimulation of a strong Th1 immune response via *Leishmania major* infection before the onset of or early in the progression of LP-BM5 infection inhibits the development of murine AIDS symptoms (27). Administration of TCR Vβ CDR1 peptide both before and after infection significantly prevents the murine retrovirus-induced suppression of IL-2 and IFN-γ secretion, while the control peptide, MCG3, from the λ L chain V region of Ig may not. IL-2 is an important growth factor for T cells, and its increased release after TCR Vβ CDR1 administration is in accordance with restored T cell proliferation, as is the loss of IL-2 secretion by cells from mice progressing to severe pathology during murine retrovirus infection (14, 15). IFN-γ has multiple distinct biologic activities, including antiviral activity, activating phagocytosis of macrophages and neutrophil cells, and stimulation of cytotoxicity by NK cells and cytotoxic T lymphocytes (CTL) (28). Thus, increased IFN-γ caused by TCR Vβ CDR1 peptide prevents the development of suppressed cell-mediated immunity in murine AIDS. Increased production of IFN-γ by TCR Vβ CDR1 peptide is also in agreement with the enhancement of NK cell activity by the peptide.

However, IFN-γ inhibits Th2 cytokine secretion, which usually is elevated during the progression of the retrovirus infection. This is supported by our findings that administration of TCR Vβ CDR1 peptide in murine AIDS significantly reduces retrovirus-induced elevation of IL-5, IL-6, and IL-10 production and IgG production, while a control peptide does not. Increased production of IFN-γ by TCR Vβ CDR1 peptide administration after retrovirus infection is also in agreement with the enhancement of NK cell activity by the peptide. Taken together, the prevention of imbalanced Th1 and Th2 cytokine production by TCR Vβ CDR1 peptide administration contributes to the normalization of the entire immune response, thereby retarding the development of immune dysfunction during murine retrovirus infection. These findings are also supported by increased $CD8^+$ with selected Vβs (31) and AAb to HIV (32), which react with some Vβs. The dose of peptide that produces optimal slowing of development of immune dysfunction during murine retrovirus infection (200 μg/mouse or approximately 10 mg/kg of body weight) is the same dose found to be optimal in humans with an autoimmune disease (26). The two adjuvants tested enhanced the effectiveness of low doses of a TCR Vβ peptide. Thus, immunization may be involved with the production of AAb (24) or cellular immunity.

In vivo activated B cells and macrophages from HIV patients produce high levels of IL-6 and TNF-α (2), as do LPS-stimulated splenocytes and peritoneal macrophages for LP-BM5 retrovirus-infected mice (14). An elevated level of TNF-α may be involved with lipid metabolism, inducing hypertriglyceridemia (28) and loss of vitamin E (29) and increased lipid peroxidation (28) during the development of murine AIDS. Elevated levels of TNF-α have also been associated with the stimulation of HIV replication in macrophages/monocytes and T cells (30). Thus, reduction of elevated levels of TNF-α in murine retrovirus infection by TCR Vβ CDR1 peptide administration ameliorates pathologic symptoms of the host induced by retrovirus infection. Peptide administration also largely prevented the loss of tissue vitamin E (R. R. Watson and B. Liang, unpublished observations).

Increased IL-6 production may explain the hyper-γ-globulinemia and global B-cell dysfunction seen with both pathogens (40). IFN, vitamin E and their combined administration significantly normalized the increased production of IL-6 by LPS-stimulated splenocytes from retrovirus-infected mice. As IL-6 and other Th2 cytokines are required to maintain hyper-γ-globulinemia and global B-cell dysfunction of murine AIDS, their reduced production, or altered restoration of IFN or vitamin E, would prevent excessive B-cell activity, including eventual B-cell lymphoma. IL-6 also governs the production of acute-phase reactants by hepatocytes and their tissue damages (28). Elevated levels of IL-6 have been associated with the stimulation of HIV replication in macrophages and T cells (30, 41). Thus, normalization of elevated levels of IL-6 by IFN and vitamin E should ameliorate pathological symptoms initiated by the murine retrovirus, explaining the partial normalization of spleen weight.

Peptide administration did not significantly prevent lymphadenopathy and splenomegaly, although there was a tendency toward less pronounced splenomegaly in specifically treated animals. Thus, TCR Vβ CDR1 peptide treatment may not ameliorate all symptoms following from retrovirus infection in the murine AIDS (MAIDS) model, such as prevention of deaths from asphyxiation by enlarged lymph nodes. However, conclusive observations may not yet be apparent in this case, given the statistical error in measurement of spleen size.

In addition, administration of a single Vβ TCR peptide did not totally prevent immune dysfunction. AAbs were maintained in high levels to two of seven Vβ peptide families

tested (24), suggesting that several T cell families or clones were stimulated by the retrovirus infection. Deletion of some Vβ genes significantly delayed arthritis onset (33). This suggests that the prevention of immune dysfunction by peptide administration in retrovirus-induced immunodeficiency syndrome (MAIDS) may be due to modulation of a T cell subset. As the spleen weight and lymph node size were not significantly reduced, we would still expect deaths due to asphyxiation, with enlarged lymph nodes, while maintaining near normal infectious disease resistance. Complete maintenance of normal immune function may be possible by preventing stimulation of all clones induced by the murine retrovirus Ag by administration of the Vβ peptides from the several TCR clones stimulated by murine retrovirus infection.

In experiments with the murine MAIDS model, administration of TCR Vβ CDR1 in saline by intraperitoneal injection either before (prophylaxis) or after (therapeutic) infection with the LP-BM5 MuLV retrovirus modulates retrovirus-induced immune dysfunction and cytokine dysregulation of T-cell function. In the prophylactic model, TCR Vβ CDR1 peptide was administered twice, on days −7 and −3 before retrovirus infection. In the therapeutic model, the mice were administered peptide on days 10 and 14 post-infection. Doses of peptide ranging from 0 to 500 μg/mouse were administered, in the presence or absence of adjuvants such as poly (AU) or RIBI adjuvant. An effective dose in both cases was 200 μg/mouse administered intraperitoneally in saline, although the addition of adjuvant enabled the use of less peptide in certain cases. For example, doses of 5 and 25 μg/mouse administered in the presence of adjuvant improved both the capacity for B and T cell mitogenesis in retrovirally infected mice.

The preferred dose of 200 μg/mouse in saline administered by intraperitoneal injection corresponds to approximately 10 mg/kg of body weight as a preferred dosage to be extrapolated for human usage, given that the body weight of a mouse is approximately 20 g. Dosage amounts, however, may vary depending on the route of administration and depending on whether the TCR Vβ CDR1 peptide is administered with or without adjuvant. For example, for subcutaneous administration by injection, less than 1 mg/kg of body weight may be administered for the peptide therapy to be effective. Preferably, peptide administered to a host infected with an immunodeficiency-type retrovirus should be made in at least two administrations, and multiple doses administered approximately once per month may increase efficacy.

Administration early or with a significant amount of TCR Vβ CDR1 peptide may be necessary to prevent immune dysfunction, and administration early in the infection, prior to significant immune dysfunction, may be critical. When administered shortly after LP-BM5 infection, the TCR Vβ CDR1 peptide largely, but not totally, maintains normal cytokine production. Preservation of immune function occurs similarly in mice that are administered the peptide before as well as after retrovirus infection. The greater the elapsed time post-infection before peptide administration, the greater the immune dysfunction that develops. Thus, TCR Vβ CDR1 peptide administration prevents immune dysfunction rather than restores it. However, a control peptide from a λ L chain CDR1 has no effect on preventing retrovirus-induced immune deficiency. The addition of adjuvant has a variable effect expanding the efficacy of very low (otherwise ineffective) doses of TCR antigen. TCR Vβ CDR1 peptide functions as an immunoregulatory element in the complex networks of interactions among the components of the immune system and anti-oxidation system.

In the murine model, the concentrations of hepatic and cardiac vitamin E are significantly ($P<0.05$) reduced by retrovirus infection (36), while TCR Vβ CDR1 peptide administration, at dosages of about 200 μg/mouse and 4-weeks postinfection, significantly ($P<0.05$) maintains hepatic and cardiac vitamin E levels at or near those of uninfected mice (36). Retrovirus infection significantly ($P<0.05$) increases hepatic and cardiac lipid peroxidation that produces more free radical products, i.e., lipid fluorescence and diene conjugates (36). TCR Vβ CDR1 peptide administration, at dosages of 100–500 μg/mouse and 4-weeks postinfection, significantly ($P<0.05$) reduces the hepatic and cardiac-free radical products (36).

The study of TCR Vβ CDR1 peptide provides an insight into the pathogenesis during progression to AIDS, as well as into the mechanisms of idiotypic networks involving AAb and autoreactive T cells as regulatory elements. These results also expand understanding of the roles of vitamin E and free radical products on regulation of immune function during retrovirus infection, which support the concept that combination of immune therapy and vitamin E supplementation therapy for murine retrovirus infection may be more efficacious than either alone.

In summary, a TCR Vβ CDR1 peptide is a potentially immunomodulating agent that achieves its immune system-enhancing effects through indirect mechanisms, possibly by preventing selective expansion of TCR Vβ T cells induced by the chronic retroviral Ag exposure. These findings help to evaluate the mechanisms contributing to retrovirus-induced immunodeficiency and to learn how to prevent their functioning. TCR Vβ CDR1 peptide administration may provide long term prevention of retrovirus-induced immune dysfunction. In addition, the use of TCR Vβ CDR1 peptide may be important in forestalling initial episodes of general immune disorders in some patients by extending the period between retrovirus infection and the appearance of immune deficiencies.

EXAMPLE 1

Animals and Murine AIDS

Female C57B1/6 mice, 5 wk old, were obtained from Charles River Laboratories (Wilmington, Del.). Animals were cared for as required by the University of Arizona Committee on Animal Research. After 2 wk in the animal facility at the Arizona Health Sciences Center, the mice were randomly assigned to one of the following six treatments: (1) uninfected normal mice injected with saline (pyrogen free); (2) uninfected normal mice injected with MCG3 (a synthetic peptide containing the CDR1 sequence of MCG-λ L chain) control peptide; (3) uninfected normal mice injected with TCR Vβ CDR1; (4) LP-BM5-infected mice injected with saline; (5) LP-BM5-infected mice injected with control peptide (MCG3); and (6) LP-BM5-infected mice injected with TCR Vβ CDR1 peptide. Group A was comprised of the three uninfected groups, and group B was comprised of the groups infected with LP-BM5.

Administration of peptides (200 μg/mice in saline, i.p.) was performed twice, on days −7 and −3 before retrovirus infection. After 42 days of retrovirus infection, the mice were sacrificed for immunologic analysis. In other experiments, the peptides and saline were injected on days 10 and 14 postinfection. In the dose-response experiment, mice were immunized with different doses of TCR Vβ peptide (0, 5, 25, 100, 200, and 500 μg/mouse, i.p.) on day 10 postinfection. Only in the adjuvant experiment were adjuvants, poly(AU) and Ribi, mixed with the peptide before injection. The mice were sacrificed 14 wk postinfection. The

retrovirus was administrated i.p. to mice on 0.1 ml with an ecotrophic (XC) titer of 4.5 $\log_{10}$ PFU/ml, which induces disease with a time course comparable to that previously reported (1). Uninfected normal mice were injected with complete culture medium used for LP-BM5 virus growth as controls. Infection of adult female C57B1/6 mice with LP-BM5 murine leukemia leads to the rapid induction of clinical symptoms with virtually no latent phase.

Peptides

A set of overlapping 16-mer peptides that duplicate the covalent structure of the VβDβJβCβ protein (15, 16) predicted from a human TCR Vβ gene sequence (17) was produced. TCR Vβ CDR1 that corresponds to the completed CDR1 and N-terminal five residues of Fr2 (15, 18) of the human Vβ8.1 gene product (17)has the sequence Cys Lys Pro Ile Ser Gly His Asn Ser Leu Phe Trp Tyr Arg Gln Thr (SEQ ID NO:1). As a control peptide, we used a 16-mer corresponding to the CDR1 of the L chain MCG (19), because the LP-BM5-infected mice did not produce AAb to this peptide. Its sequence is Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser Trp Tyr (SEQ ID NO:2).

The peptide preparations were free of endotoxin. Normal polyclonal IgG pools contain natural AAb against peptide segments corresponding to CDR1, Fr3, and a constant region loop peptide of the TCR β-chain (15). Unimmunized mice also have natural IgG Ab directed against the same peptide segments; in particular, there is strong reactivity to the human CDR1 test peptides. A computer comparison of human and murine Vβ sequences (S. F. Schulter and J. J. Marchalonis, unpublished analysis) using the progressive alignment algorithm of Feng and Doolittle (20) showed that certain human and murine Vβ sequences could be grouped into families, e.g., human Vβ6 and Vβ8 correspond to murine Vβ11 with human and murine Vβ5 in the same clusters.

Standard cytokines and their antibodies

Rat anti-murine IFN-γ mAb, standard rIFN-γ, hamster anti-TNF-α mAb, standard rTNF-α, rabbit anti-murine TNF-α serum, rat anti-murine IL-6 mAb, and murine IL-6 were obtained from Genzyme (Boston, Mass.). Rat anti-murine IL-5 and IL-10 Ab, biotin-rat anti-murine IL-5 and IL-10 mAb, and murine rIL-5 and rIL-10 were obtained from Pharmingen (San Diego, Calif.). Goat anti-murine IL-6 polyclonal Ab was obtained from R&D System (Minneapolis Minn.). Rabbit anti-murine IFN-γ antiserum was prepared in our laboratory.

ELISA for AAb to TCR-β and MCG3

The specific titers of serum IgG to TCR-β and MCG3 were determined by ELISA as previously described (15, 16, 18, 19). Briefly, peptides were dissolved in carbonate buffer, pH 9.6 at 1 μg/well onto Nunc Maxisorb (Nunc. Roskilde, Denmark) 96-well microtiter plates and dried down at 37° C. overnight. The plates were washed with PBST (PBS and 0.05% Tween 20). Nonspecific binding was blocked by incubation with PBSTFG (PBST plus 1.0% fish gelatin). PBSTFG was used as diluent for all sera and conjugated Ab. The plates were washed three times with PBST and incubated for 1.5 h with diluted serum. The plates were washed three times and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG H and L chains (Jackson Immunoresearch, West Grove, Pa.) at a dilution of 1/2000 for 1 h at room temperature. To detect the presence of serum IgG bound to peptide-coated wells, plates were washed three times with PBST and developed with ABTS (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M citrate buffer, pH 4.0. The absorbance at 405 nm was measured after 1 h with a Titertek Multiskan Plus ELISA plate reader EFLABor by Lab Systems and Flow Labs, Finland. Serial twofold dilutions were made for each serum in duplicate with a standard dilution of 1/200. Background binding was measured as the binding of each serum to PBSTFG-blocked microtiter wells in the absence of Ag. The results are represented as the mean titer for each treatment group. The titer for each individual serum was calculated as the inverse dilution at which an absorbance of 0.25 at 405 nm was measured. To allow statistical analysis, a numeric value was required to represent sera that had an absorbance of less than 0.25 at the initial dilution of 1/200. Therefore, sera with no detectable titer were assigned a value of 25, since in our assay, at a dilution of 1/25 most of the sera would have an absorbance close to 0.25. The analysis of variance was conducted using the cell means model: $y_{ijk}=mean_{ij}+e_{ijk}$, where y_{ijk} is the response for the k^{th} individual who received the j^{th} infection and the i^{th} treatment, means $_{ij}$ is the means response for the ij^{th} group, and e_{ijk} are the random errors associated with the ijk^{th} individual. The e values are assumed to be independent of one another and, given retrovirus status, to have the same variance. For purposes of statistical inference, the e values are also assumed to be normally distributed. The test we used is based on the usual two-sample t-static, but adjusts the degrees of freedom according to the minimum of the sample sizes involved. An adjustment involving bootstrapping the residual errors was made to resolve the issue of multiple comparisons.

ELISA for cytokines

IL-2, IFN-γ, IL-5, and IL-10 were produced by splenocytes as described previously (21). Briefly, spleens were gently teased with forceps in culture medium (RPMI 1640 containing 10% fetal calf serum, 2mM glutamine, and 100 U/ml penicillin and streptomycin), producing a single cell suspension of spleen cells. Red blood cells were lysed by the addition of a lysis buffer (0.16 M ammonia chloride Tris buffer, pH 7.2) at 37° C. for 2 min. Then, the cells were washed twice with culture medium (CM). The cell concentration was counted and adjusted to 1x10 cells/ml. Splenocyte viability was more than 95%, as determined by trypan blue exclusion. Splenocytes (0.1 ml/well; 1×10^7/ml) were cultured in triplicate on 96-well flat bottom culture plates (Falcon, Lincoln Park, N.J.) with CM. Splenocytes were then stimulated with 10 μg/ml concanavalin A (Con A) (0.1 ml/well: Sigma Chemical Co.) for induction of IL-2 and IL-10 with 24-h incubation and for induction of IL-5 and IFN-γ with 72-h incubation at 37° C. in a 5% CO_2 incubator. Splenocytes were also stimulated by lipopolysaccharide (LPS, 5 μg/ml: Life Technologies, Grand Island, N.Y.) for 24 h to induce IL-6 and TNF-α production.

After incubation, the plates were centrifuged for 10 min at 800xg. Supernatant fluids were collected and stored at −70° C. until analysis. They were determined by sandwich ELISA as described previously (21).

Mitogenesis of splenocytes

Splenic T and B cell proliferation was determined by [^{3}H]-thymidine incorporation as described previously (21). Briefly, splenocytes in 0.1 ml of CM (1×10^7/ml) were cultured in 96-well flat bottom culture plates (Falcon) with Con A and LPS (5 μg/ml) and CM. They were incubated at 37° C. in a 5% $C0_2$ incubator for 20 h for Con A-induced T-cell proliferation and for 44 h for LPS-induced B cell proliferation, and then pulsed with [^{3}H]-thymidine (0.5 μCi/well: New England Nuclear, Boston, Mass.).

After 4 h, they were harvested by a cell sample harvester (Cambridge Technology, Cambridge, Mass.). Radioactivity was determined by a liquid scintillation counter (Tri-Carb, 2200CA, Packard, Laguna Hills, Calif.). Data are presented as counts per min.

Natural Killer (NK) cell cytotoxicity

NK cell function was measured by a fluorescent concentration release assay modified from the method of Wierda et al. (22) Briefly, this method measures the fluorescent dye 2,7'-bis-(carboxyethyl)5,6'-carboxyfluorescein (Molecular Probes, Eugene, Oreg.,) remaining in the target cells using the Pandex Fluorescence Concentration Analyzer (IDEX, Portland, Me.). YAC-1 target cells were washed once with PBS and labeled with the carboxyfluorescein derivative. Effector to target (E:T) ratios were adjusted to 100:1 and 50:1, and cells were plated in U-bottom microtiter plates (Falcon 3077, Becton Dickinson, Rutherford, N.J.) containing 4×10^4 target cells/100 μl. The plate was centrifuged (90×g) for 3 min to facilitate cell to cell interaction. The cells were then incubated at 37° C. in a humidified atmosphere of 5% CO_2 for 3 h.

After incubation, 20 μl of 1% inert fluoricon polystyrene assay particles were added to each well of the plate (Pandex Harvesting Plate, IDEXX Research Products Div., Westbrook, Me.), and an 80-μl aliquot from each well of the irradiation plate was transferred to a Pantex plate. The epifluorescence of each well in the harvest plate was

automatically read at 485/533 nm excitation/emission wavelengths for 2,7'-bis-(carboxyethyl) 5,6'-carboxyfluorescein using the Pandex Fluorescence Concentration Analyzer. Specific cytotoxicity (percentage) was calculated as follows:

$$\frac{\text{Spontaneous Release} - \text{Experimental Fluorescence}}{\text{Spontaneous Release} - \text{Maximum Release}} \times 100$$

ELISA for IgG detection

Splenocytes (1×10^7/ml) from mice in the same experimental group were individually cultured with CM in triplicate on 96-well tissue culture plates (0.1 ml/well; Falcon). Then, splenocytes were stimulated with LPS (Life Technologies; 10 μg/ml, 0.1 ml/well) diluted in CM. After 72-h incubation at 37° C. in a 5% CO_2 incubator, the plates were centrifuged for 10 min at 800×g, and supernatant fluids were collected and stored at −70° C. until analysis. Murine IgG production was determined by sandwich ELISA as described previously (21).

Lymphocyte subpopulation measurement of IL-2R$^+$ cells

Mouse spleens were individually collected in complete RPMI 1640 medium, and mononuclear cells were obtained by gently teasing with tweezers. Cell suspensions were washed with medium, and red blood cells (RBC) were lysed in an ammonium chloride solution. The remaining cell suspensions were washed once with cold medium and counted with cosin Y to prepare the desired viable cell concentrations (1–2 million/0.1-ml tube) for lymphocyte surface marker determinations. The numbers of cells expressing IL-2R were determined using rat IgG2a mAb (clone AMT-13) obtained from Boehringer Mannheim (Indianapolis, Ind.) as described previously (23).

Statistics

All parameters were compared between all groups using one-way ANOVA followed by Duncan's multiple range test between any two groups. $p<0.05$ was considered a significant difference between two groups.

Results

Production of AAb to TCR-β and MCG3

Comparison of mean titers of Ab binding TCR Vβ CDR1 peptide (group A—uninfected vs. group B—infected with LP-BM5) indicates that retrovirus infection of C57BL/6 female mice results in a highly significant increase ($p<0.0005$) in specific Ab titers in contrast to that in medium-injected controls. This phenomenon has been regularly observed, and the anti-TCR Ab profile following LP-BM5 infection has been previously characterized. Treatment of retrovirally infected mice with the TCR Vβ CDR1 peptide did not result in a significant increase in mean Ab titers compared with either retrovirally infected, saline-treated mice ($p=0.17$) or retrovirally infected, control peptide-treated mice ($p=0.07$). When sample size is considered, the mean Ab titers of the retrovirally injected TCR Vβ CDR1 peptide-treated group and the retrovirally injected, control peptide-treated group are marginally different. ELISA binding assays did not detect any positive titers to the MCG3 peptide or to blank (background) plates.

Cytokine production

In vitro production of Th1 cytokines, IL-2 and IFN-γ, by Con A-stimulated splenocytes was significantly ($p<0.05$) inhibited in the retrovirus-infected mice. A TCR Vβ peptide was selected because high levels of AAb were induced by the retroviral infection (24, 25). TCR Vβ administration before infection significantly ($p<0.05$) normalized IL-2 and IFN-γ release by Con A-stimulated splenocytes, while administration of the control peptide, MCG3 did not. Release of Th2 cytokines, IL-5 and IL-10, was significantly ($p<0.05$) increased in retrovirus-infected mice. TCR Vβ administration before infection significantly reduced IL-5 and IL-10 release by Con A-stimulated splenocytes, while administration of the control peptide MCG3 did not. Production of TNF-α by LPS-stimulated splenocytes was elevated in the retrovirus-infected mice. TCR Vβ administration significantly ($p<0.05$) inhibited TNF-α release.

TABLE 1

Mean ELISA titers against the target TCR Vβ CDR1 peptide

Treatment Groups	N	Mean	Min/Max	S.D.
Group A (total uninfected)	20	326	25/1150	375
Medium + saline	8	418	25/1150	447
Medium + MCG3	4	443	25/1150	795
Medium + TCR	8	175	25/500	182
Group B (total infected)	24	1398	25/4500	1135
LP-BM5 + saline	8	1197	25/2800	1156
LP-BMT + MCG3	8	921	25/1800	597
LP-BM5 + TCR	8	2075	25/4500	1308

Individual sera were screened by ELISA for binding to each antigen with a starting dilution of 1:2000 followed by serial twofold dilutions. A titer of 25 was assigned to any serum with an undetectable titer in order to allow statistical analysis of mean values.

Immune responses

NK cell activity and T and B cell proliferation in response to mitogens were significantly ($p<0.05$) suppressed in the retrovirus-infected mice. TCR Vβ peptide administration before infection significantly ($p<0.05$) normalized the suppressed NK cell activity and 7 cell proliferation, while administration before infection of the control peptide MCG3 did not. TCR Vβ peptide administration before infection prevented the retrovirus-induced suppression of B cell proliferation, but this was not statistically significant.

Murine AIDS symptoms

Hypergammaglobulin production is an important indicator of early retrovirus infection during the progression to murine AIDS. IgG production by LPS-stimulated splenocytes was significantly ($p<0.05$) increased in the retrovirus-infected mice. TCR Vβ administration before infection significantly ($p<0.05$) reduced IgG production, while admin-

istration of the control peptide MCG3 did not. Mice were injected with saline, the control peptide (MCG3), or Vβ 8.1 2 and 6 wk post-LP-BM5 infection.

Dose response and adjuvant stimulation

As others found that TCR peptide immunization with high and low doses was less effective at preventing immune dysfunction in autoimmune disease (26), we investigated the actions of different doses of the TCR Vβ peptide on immune dysfunction. The effects of different doses of peptide injected after retrovirus infection on B and T cell mitogenesis in vitro were determined. Doses of 5, 25, and 100 μg/mouse of Vβ peptide prevented only some loss of B cell mitogenesis and did not prevent loss of T cell mitogenesis 16 wk after retrovirus infection when murine AIDS had developed. However, the higher doses (200 μg/mouse, used in all other experiments, as well as 500 μg/mouse) increased both B and T cell mitogenesis. Adjuvants might enhance the effectiveness of Vβ peptide doses that were too low to prevent immune dysfunction. When the TCR Vβ peptide was injected with either of two adjuvants, poly(a)U or Ribi, B cell proliferation in vitro was higher than that in mice injected with the peptide only or saline. T cell mitogenesis was similarly affected at 16 wk of retrovirus infection when murine AIDS had appeared in untreated infected mice.

Immunomodulation in mice immunized with TCR peptide after retrovirus infection

As Vβ peptide immunization postinfection would indicate whether the peptide treatment was normalizing immune dysfunction or preventing its development, mice were immunized at various times postinfection. Injection of the TCR Vβ peptide after retrovirus infection largely prevented the loss of Th1 cytokines, IL-2 and IFN-γ, produced by splenocytes stimulated in vitro with mitogens. Th2 cells were significantly prevented from developing their increased output of the cytokines, IL-5 and IL-6, by TCR Vβ, but not control peptide, immunization performed after retrovirus infection. Immunization after retrovirus inoculation also prevented the loss of T and B cell mitogenesis and the decline in NK cell activity, and somewhat prevented an increase in activated T cells, IL-2R$^+$. Injection of the Vβ peptide after infection also prevented elevation of monocyte cytokine production of IL-6 and TNF as well as IgG synthesis in vitro. However, it did not significantly reduce spleen size. Immunization with the Vβ peptide at increasingly longer times postinfection than 2 wk prevented less of the loss of B cell mitogenesis in vitro.

EXAMPLE 2

Animals and Murine AIDS

Female C57BL/6 mice, 4-weeks old, were obtained from Charles River Laboratories Inc. (Wilmington, Del.). Animals were cared for as required by the University of Arizona Committee on Animal Research. After 2 weeks housing in the animal facility in the Arizona Health Science Center (Tucson, Ariz.), they were randomly assigned to one of the following treatments with 8 mice per group for study A: (1) uninfected, normal mice; (2) uninfected, normal mice injected with saline (pyrogen free); (3) LP-BM5-infected mice injected with 5 μg TCR Vβ CDRI peptide; (4) LP-BM5infected mice injected with 25 μg TCR Vβ CDR1 peptide; (5) LP-BM5 infected mice injected with 100 μg TCR Vβ CDR1 peptide; (6) LP-BM5-infected mice injected with 200 μg TCR Vβ CDR1 peptide; (7) LP-BM5-infected mice injected with 500 μg TCR Vβ CDR1 peptide; (8) LP-BM5-infected mice injected with saline and 200 μg Poly AU adjuvant; (9) LP-BM5-infected mice injected with 25 μg TCR Vβ CDR1 peptide and 230 μg Poly AU adjuvant; (10) LP-BM5-infected mice injected with saline and 230 μg Ribi MPL TDM CWS adjuvant; (11) LP-BM5-infected mice injected with 5 μg TCR Vβ CDR1 peptide and 230 μg Ribi MPL TDM CWS adjuvant; and (12) LP-BM5-infected mice injected with 25 μg TCR Vβ CDR1 peptide and 230 Ribi MPL TDM CWS adjuvant.

LP-BM5 retrovirus was administered intraparitoneally to mice in 0.1 ml saline with an exotropic titre (XC) of 4.5 $\log_{10}$ plaque-forming units (PFU)/ml, which induces disease with a time course comparable to that previously published (1). Administration of peptides (dissolved in saline) and adjuvants were performed 2 weeks after LP-BM5 infection. Uninfected, normal mice were injected with complete culture medium used for LP-BM5 virus growth as controls. Infection of adult female C57BL/6 mice with LP-BM5 MuLV leads to the rapid induction of clinical symptoms with virtually no latent phase.

For study B, the treatment groups were: (1) LP-BM5-infected mice injected with saline 2 weeks after infection; (2) LP-BM5-infected mice injected with 200 μg TCR Vβ CDR1 peptide 2 weeks after infection; (3) LP-BM5-infected mice injected with 200 μg TCR Vβ CDR1 peptide 4 weeks after infection; (4) LP-BM5-infected mice injected with 200 μg TCR Vβ CDR1 peptide 6 weeks after infection; (5) LP-BM5-infected mice injected with 200 μg TCR Vβ CDR1 peptide 8 weeks after infection; and (6) LP-BM5-infected mice injected with 200 μg TCR Vβ CDR1 peptide 10 weeks after infection.

Peptides

A set of overlapping 16-mer peptides that duplicate the covalent structure of the VβDβJβCβ protein (15, 35) predicted from a human TCR Vβ gene sequence (17) was produced. TCR Vβ CDR1 that corresponds to the completed CDR1 and N-terminal five residues of Fr2 (15, 18) of the human Vβ8.1 gene product (17) has the sequence Cys Lys Pro Ile Ser Gly His Asn Ser Leu Phe Trp Tyr Arg Gln Thr (SEQ ID NO:1). Normal polyclonal IgG pools contain natural AAb against peptide segments corresponding to CDR1, Fr3, and a constant region loop peptide of the TCR β-chain (15). Unimmunized mice also have natural IgG Ab directed against the same peptide segments; in particular, there is strong reactivity to the human CDR1 test peptides. A computer comparison of human and murine Vβ sequences (S. F. Schulter and J. J. Marchalonis, unpublished analysis) using the progressive alignment algorithm of Feng and Doolittle (20) showed that certain human and murine VP sequences could be grouped into families, e.g., human Vβ6 and Vβ8 correspond to murine Vβ11 with human and murine Vβ5 in the same clusters.

Standard Cytokines

Rat anti-murine IFN-γ, IL-2-, IL-6-, IL-10-purified antibodies, rat anti-murine IFN-γ, L-2-, IL-6-, IL-10-biotinulated antibodies, and recombinant murine IFN-γ, IL-2-, IL-6-, IL-10 were obtained from Pharmingen (San Diego, Calif.).

ELISA for cytokines

IL-2, IFN-γ, IL-6, and IL- 10 were produced by splenocytes as described previously (21). Briefly, spleens were gently teased with forceps in culture medium (RPMI 1640 containing 10% fetal calf serum, 2mM glutamine, and 100 U/ml penicillin and streptomycin), producing a single cell suspension of spleen cells. Red blood cells were lysed by the addition of a lysis buffer (0.16 M ammonia chloride Tris buffer, pH 7.2) at 37° C. for 3 min. Then, the cells were washed twice with culture medium (CM). The cell concentration was counted and adjusted to 1×10^7 cells/ml. Splenocyte viability was more than 95%, as determined by trypan blue exclusion. Splenocytes (0.1 ml/well; 1×10^7/ml)

were cultured in triplicate on 96-well flat bottom culture plates (Falcon, Lincoln Park, N.J.) with CM. Splenocytes were then stimulated with 10 μg/ml concanavalin A (Con A) (0.1 ml/well: Sigma Chemical Co.) for induction of IL-2 and IL-10 with 24-h incubation and for induction of IL-5 and IFN-γ with 72-h incubation at 37° C. in a 5% CO_2 incubator. Splenocytes were also stimulated by lipopolysaccharide (LPS, 10 μg/ml: Gibco, Grand Island, N.Y.) for 24 h to induce IL-6 production. After incubation, the plates were centrifuged for 10 min at 800×g. Supernatant fluids were collected and stored at −70° C. until analysis. They were determined by sandwich ELISA as described previously (21).

Mitogenesis of splenocytes

Splenic T and B cell proliferation was determined by [^{3}H]-thymidine incorporation as described previously (21). Briefly, splenocytes in 0.1 ml of CM (1×10^7/ml) were cultured in 96-well flat bottom culture plates (Falcon) with Con A and LPS (10 μg/ml). They were incubated at 37° C. in a 5% CO_2 incubator for 20 h for Con A-induced T-cell proliferation and for 44 h for LPS-induced B cell proliferation, and then pulsed with [^{3}H]-thymidine (0.5 μCi/well: New England Nuclear, Boston, Mass.). After 4 h, they were harvested by a cell sample harvester (Cambridge Technology, Cambridge, Mass.). Radioactivity was determined by a liquid scintillation counter (Tri-Carb, 2200CA, Packard, Laguna Hills, Calif.). Data was presented as counts per minute.

Natural Killer (NK) cell cytotoxicity

NK cell function was measured by a fluorescent concentration release assay modified from the method of Wierda et al. (22) Briefly, this method measures the fluorescent dye 2,7'-bis-(carboxyethyl)5,6'-carboxyfluorescein (Molecular Probes, Eugene, Oreg.) remaining in the target cells using the Pandex Fluorescence Concentration Analyzer (IDEX, Portland, Me.). YAC-1 target cells were washed once with PBS and labeled with the carboxyfluorescein derivative. Effector to target (E:T) ratios were adjusted to 100:1 and 50:1, and cells were plated in U-bottom microtiter plates (Falcon 3077, Becton Dickinson, Rutherford, N.J.) containing 4×10^4 target cells/100 μl. The plate was centrifuged (90×g) for 3 min to facilitate cell to cell interaction. The cells were then incubated at 37° C. in a humidified atmosphere of 5% CO_2 for 3 h. After incubation, 20 μl of 1% inert fluoricon polystyrene assay particles were added to each well of the plate (Pandex Harvesting Plate, IDEXX Research Products Div., Westbrook, Me.), and an 80-μl aliquot from each well of the irradiation plate was transferred to a Pantex plate. The epifluorescence of each well in the harvest plate was automatically read at 485/533 nm excitation/emission wavelengths for 2,7'-bis-(carboxyethyl) 5,6'-carboxyfluorescein using the Pandex Fluorescence Concentration Analyzer. Specific cytotoxicity (percentage) was calculated as follows:

$$\frac{\text{Spontaneous Release} - \text{Experimental Fluorescence}}{\text{Spontaneous Release} - \text{Maximum Release}} \times 100$$

Statistics

The statistic tests for comparison among groups were finished in NCSS program (Kaysville, Utah) using Friedman's Block/Treatmnent test, followed by Duncan's Multiple Range Test between any two groups. $P<0.05$ was considered a significant difference between two groups.

Results

Body weight

There was no change in food consumption because of infection or injection. The body weight of the mice was not affected by various levels of TCR Vβ CDR1 peptide injection or time course of injection postinfection. The spleen and lymph node weights were significantly ($P<0.05$) elevated in the infected mice, which indicated that infection had progressed to murine AIDS.

Mitogenesis of splenocytes

Proliferation of Con-A and LPS-induced splenocytes was significantly decreased ($P<0.05$) by murine retrovirus infection. Suppression of T- and B-cell proliferation in the spleen, induced by retrovirus infection, was significantly ($P<0.05$) prevented by TCR Vβ CDR1 peptide injection. Peptide dosages above 200 μg/mouse and injection before 4 weeks postinfection maintained near normal T- and B-cell proliferation which were significantly ($P<0.05$) higher than that in infected, unimmunized mice. Injection with less than 200 μg/mouse of peptide or at 6–10 weeks postinfection did not prevent development of decreased, in vitro proliferation of mitogen-stimulated T and B cells.

Nature killer (NK) cell cytotoxicity

Murine retrovirus infection significantly ($P<0.05$) reduced the splenic NK cell activity, which was largely ($P<0.05$) maintained in infected mice injected with the TCR Vβ CDR1 peptide. Peptide dosages above 100 μg/mouse and injection by 4 weeks postinfection maintained near-normal NK cell activity which was significantly ($P<0.05$) higher than that in infected, unimmunized mice. Injection with less than 100 μg/mouse at or after 6-weeks postinfection permitted development of a significantly decreased NK cell cytotoxicity.

Influence of adjuvants on TCR immunization

The adjuvants used generally had no significant ($P>0.05$) effect on maintaining the immune function.

Cytokine production of splenocytes

In vitro production of Th1 cytokines, IL-2 and IFN-γ by Con A-stimulated splenocytes was significantly ($P<0.05$) inhibited in the retrovirus-infected mice. TCR Vβ CDR1 peptide injection significantly ($P<0.05$) normalized IL-2 and IFN-γ release by mitogen-stimulated splenocytes compared with infected, unimmunized mice. Injection with 200 μg/mouse or higher dose of peptide and before 6-weeks postinfection maintained near-normal Th1 cytokine production which were significantly ($P<0.05$) higher than that in infected, unimmunized mice. Injection at dosages of less than 200 μg/mouse or after 6-weeks postinfection had significantly ($P<0.05$) decreased Th1 cytokine production.

Release of Th2 cytokines, IL-6 and IL-10 in vitro by mitogen-stimulated spleen cells, was significantly ($P<0.05$) increased in the retrovirus-infected mice. TCR Vβ CDR1 peptide injection significantly ($P<0.05$) normalized IL-6 and IL-10 release by mitogen-stimulated splenocytes. Immunization with TCR Vβ CDR1 peptide above 100 μg/mouse and 4 weeks postinfection maintained near-normal Th2 cytokine production which was significantly ($P<0.05$) lower than that of infected, unimmunized mice. Injection at dosages of less than 100 μg/mouse or after 4-weeks postinfection permitted development of a significantly ($P<0.05$) increased Th2 cytokine production.

Those skilled in the art will recognize that, while specific embodiments have been illustrated and described, various modifications and changes may be made without departing from the spirit and scope of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 2

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Lys Pro Ile Ser Gly His Asn Ser Leu Phe Trp Tyr Arg Gln Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser Trp Tyr
1 5 10 15

What is claimed is:

1. A method of modulating the immune response in a mammal infected with a C-type retrovirus or a lentivirus, comprising administering by a systemic route an amount of T-cell receptor Vβ CDR1 peptide of SEQ ID NO:1 sufficient to stimulate the production of interleukin 2 and interferon-γ, and to suppress the production of interleukin 5, interleukin 6, interleukin 10, and immunoglobulin G.

2. The method of claim 1, wherein said lentivirus comprises HIV.

3. A method of altering the immune system response of a host infected with a C-type retrovirus or a lentivirus, comprising artificially introducing a T-cell receptor Vβ CDR1 peptide of SEQ ID NO: 1 into the bloodstream or immune system by injection so as to artificially induce said immune system to stimulate production of Th1 cytokines or suppress production of Th2 derived cytokines.

4. The method of claim 3, wherein said lentivirus comprises HIV.

5. The method of claim 3, wherein said lentivirus comprises feline immunodeficiency virus.

6. A method of altering the immune system response of a host suffering from an infectious disease comprising artificially introducing a T-cell receptor Vβ CDR1 peptide of SEQ ID NO: 1 into the bloodstream or immune system by injection so as to artificially induce said immune system to stimulate production of Th1 cytokines or suppress production of Th2 derived cytokines.

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